TITLE THE EFFECTS OF BRINE ON THE INVERTEBRATE FAUNAS OF SOME INLAND WATERS.

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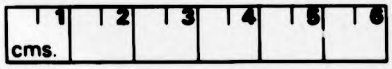

The Effects of Brine on the Invertebrate Faunas of Some Inland Waters.

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ABSTRACT

The macroinvertebrate faunas of various inland saline waters in Cheshire and N.Staffordshire were investigated. The sites included a stream and a series of ponds on a commercial brine-field, a canal receiving a brine effluent, and a number of brine springs. Salinities ranged from those of freshwater to a maximum of 117°/oo total salinity.

Approximately two hundred invertebrate species were recorded and the distributions of the more common species are discussed with reference to the salinity at which they were found and those at which they have previously been recorded. Species and groups were allocated to categories according to their range and their frequency response to increased salinities. Some of these are named as sensitive indicators of salinity.

Certain ecological changes were characteristic of increasing salinity. Principally, there were sequential changes in the groups and species of animals present. Associated with these changes was an overall decline in number of species, although diversity was assisted in moderate salinities by the appearance of species characteristic of harsh environments and of genuine brackish water species. There was also a decline and subsequent increase in total number of individuals. This was attributed to the loss of members of freshwater species as salinity increased and the successful occupation of the vacant niches by members of salt-tolerant and halobiont species.

The pattern of changes in the faunas was used to construct and validate a matrix of halobic indices whereby the severity of both poikilohaline and homoiohaline environments could be assayed.

A classification system for chloride based inland saline waters was also derived. Although the Limnetic and Oligohaline zones of marine-brackish water classification were applicable on grounds of faunal similarity, a separate system had to be formulated for inland waters of higher salinity, as they had distinctive non-marine faunas.

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alternative use of the terrestial environment and their relative impermeability. Their contribution to category II was less than 3%. The third category was composed of the remainder of the non-dipterous insects, the Plecoptera, Ephemeroptera and Trichoptera.

The Chironomidae were divided into two components. As members of the Chironominae and Tanypodinae are notable for their tolerance of salinity (Thienemann 1954) they were allocated to category IV whilst the Diamesinae and Orthocladiinae comprise category V. The final category,VI, contained the remaining Diptera.

Changes in number of species. A dramatic decrease in species abundance was apparent along the stream as salinity conditions became more extrême (fig.10). Stations 1 and 2 of the stream had the richest fauna consisting of 44-45 different species, whereas stations 3-6 had a slightly impoverished fauna of 33 to 37 species. The variety of fauna at station 7 was similar with 31 species, but there was an abrupt decline in diversity by stations 8 and 9 which had only 16 to 20 species. Stations 10 and 11 had the sparsest fauna comprised of only 14 species.

DISCUSSION

1. The freshwater stretches of the stream.

Monitoring of the physical and chemical parameters of the stream indicated that salinity was the only factor likely to affect the fauna. Temperature, water hardness, hydrogen ion concentration and oxygen saturation were all favourable and the fauna of the freshwater stretches was rich both in terms of its diversity and its composition. Use of the Trent Biological System of Stream Classification (Woodiwiss 1964) gave an index of 9, signifying a healthy stream fauna.

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CHAPTER I

INTRODUCTION

The salt deposits of the Cheshire Basin.

Rock salt deposits in Britain are confined to the Triassic strata, and in particular are found within the Keuper Series which is maximally developed in the Cheshire Basin (fig.1) though the salt-beds of Staffordshire are connected, being the most south-easterly portion of the same deposit (Sherlock, 1921). The Cheshire Basin is now bounded to the east by the extremities of the Pennine anticline in Derbyshire and Staffordshire, and to the west by the Peckforton Hills and the Delamere Range, and is thought to have been created or accentuated by earth movements in the late Carboniferous and early Permian period (Evans et al., 1968). It was in this trough that the Permo-Triassic rocks such as the Keuper sandstones and mudstones were deposited subaqueously. The sandstones are overlaid by saliferous beds which fall into two well-defined formations, the Lower Keuper Saliferous Beds and the Upper Keuper Saliferous beds with generalised thicknesses of 190m and 396m respectively (Poole and Whiteman, 1966).

The two beds are separated by approximately 30m of mudstone and are covered by glacial drift to depths of up to 340m, though towards the edges of the trough, both beds approach the surface forming "wet rock head" where solution by groundwater creates natural brine runs and causes subsidence.

The salt deposits are thickest in the area between Middlewich and Holford Moss. Evans et al.(1968) point out that the detailed sequence within the saliferous beds is remarkably constant, and apart from considerations of the sheer mass of rock-salt, the absence of dolomite and anhydrite beds (the deposition of which normally precedes the crystalization of halite) suggests that the source of the salt was highly concentrated sea-water. The same authors postulate that the

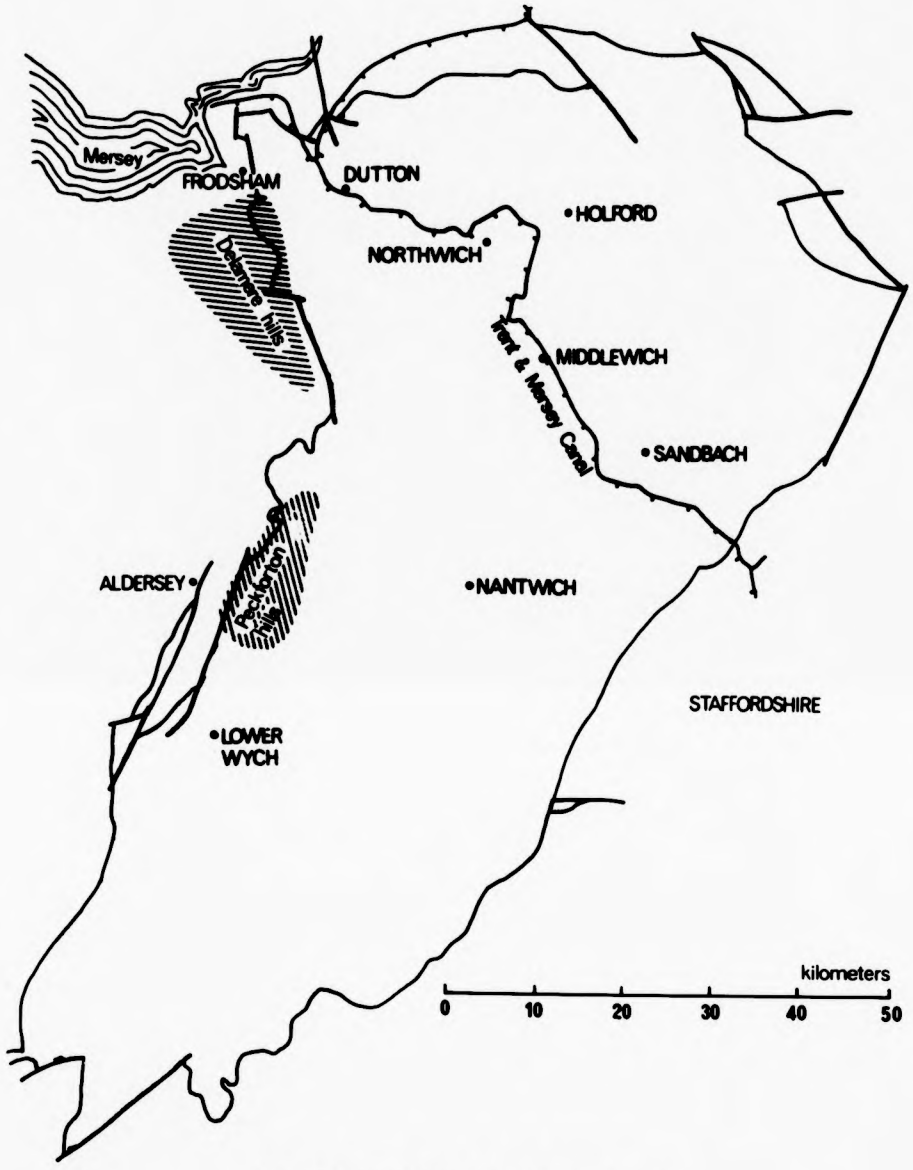


Fig. 1 The Cheshire Salt Field

Cheshire area was in continuous contact with an open sea which lay in the area now occupied by the Alps, and which covered an intervening wide, shallow shelf. A tropical climate is postulated which, by causing a high evaporation rate, would induce a steady landward flow of sea water which would first become saturated with carbonates and then with sulphates, and dolomite and anhydrite beds would be deposited. The resultant inflow reaching Cheshire would be composed primarily of concentrated sodium chloride solution, and owing to its greater density, saturated brine would be maintained in the deeper part of the basin in the Holford area, and also in another shallower basin in Staffordshire. Crystalisation of halite in such conditions would be extremely rapid, and in view of the source of the halite, present day saline waters arising from renewed solution of the deposits must be considered thalassohaline in the terminology of Bond (1935).

Exploitation of the salt deposits is of some antiquity and it has been established that the Romans worked the brine at Middlewich (Anon,1971). Many other natural brine springs have been documented historically (Calvert 1915), several of which were situated along the Weaver valley and also in the parish of Weston in Staffordshire (Sherlock 1921). In later history, pits were sunk to utilize underground brine-runs and the most southerly one on the Cheshire saltfield, the Lower Wych pit, was exploited late into the 19th century. Modern industry exploits the brine either by direct pumping from natural brine runs, or by the controlled solution of salt in underground cavities from which brine is then pumped (Collins 1969). The latter process was developed by I.C.I. and is utilised on the Holford brinefield whence over three-quarters of the country's salt is produced.

In the present study, investigations of the ecology of inland saline waters in this area were concentrated not only on natural salt springs, but also where brine has been produced by man during his exploitation of the salt deposits and where it has contaminated surface waters.

Ecological investigations in relation to salinity.

The faunas of inland saline waters characteristically differ from those of littoral-brackish waters in faunal affinity. Whereas in brackish waters a large component of the fauna is recruited from the sea, in inland saline waters the fauna is almost entirely dominated by animals of freshwater origin, and in particular by the freshwater insects, which, by virtue of their terrestial ancestry are well equipped to resist dehydration.

Although almost all ecological investigations of the effects of salinity on British faunas have been carried out in marine littoral situations, some of these, particularly studies of salt-marshes, are pertinent to the present investigation by virtue of the common insect component.

Butler and Popham (1958) studied the effects of an influx of sea water on the brackish water insect fauna of the Spurn Peninsula and concluded that approximately $9^{o}/oo$ total salinity was critical for many non-dipterous insects. Nicol (1933,1935) investigated the fauna of three salt-marshes on the Northumberland coast which included a number of freshwater colonists. Several Diptera, including Aedes detritus Haliday, Chironomus aprilinus Meigen and Eristalis sp., and also Gammarus duebeni Lillj. were recorded from a wide range of salinities. Nicol (1938) also found occasional freshwater invertebrates in the brackish lochs of Orkney. Sutcliffe (1959, 1961a) investigated the salt-marsh fauna at Seaton Sluice and found that the breeding population consisted mainly of Odonata and Diptera (Chironomidae and Ephydra riparia Meigen), though the mixohaline* caddis, Limnephilus affinis Curtis was also common. Stuart (1941) studied the chironomid fauna of littoral pools at Millport, the salinity of which ranged from that of freshwater to greater than that of sea water. He named nine species, including Cricotopus sylvestris Fabricius, which he considered to be tolerant of high salinities. Pyefinch (1937) investigated a

*The term 'mixohaline' is used to include saline waters other than true brackish waters which have a marine origin.

series of littoral pools on Bardsey Island where a stream entered the sea, and found a restricted freshwater fauna. Howes (1939) studied the ecology of a recently isolated lagoon and also found a restricted freshwater fauna composed mainly of insects. Chironomidae were common and specimens of Aedes detritus, Nemotelus sp, and the mixohaline corixid, Sigara selecta (Fieber) were also recorded. Lansbury (1954) recorded another mixohaline corixid, Sigara stagnalis (Leach) on the Kent coast.

Those British mixohaline species whose distributions have received most attention are the Gammarus spp. The brackish water Gammarus tigrinus Sexton was first described in Britain by Sexton and Cooper (1939) and subsequently, Sexton (1942) assimilated and interpreted British records of the distribution of the estuarine Gammarus zaddachi Sexton. Hynes (1954), examined the freshwater and brackish water distribution of Gammarus duebeni in Britain and later provided information on the distribution and interelationships of all the British freshwater shrimps (Hynes 1955a, b, 1956, Hynes et al 1960). Sutcliffe (1967c) re-examined some of the factors relating to the distribution of G. duebeni. Kinne (1952) investigated the biology of Gammarus duebeni and G.zaddachi, and later studied their distribution in Kiel Bay (Kinne 1954). He subsequently published ecological data on G.duebeni (Kinne 1959). Smit (1974) and Pinkster (1975) have both discussed the distribution of G.tigrinus relative to that of G.zaddachi and G.duebeni in the Netherlands.

On the Continent a wealth of investigations into brackish-water ecology have been conducted in various regions of the Baltic Sea, and in the Netherlands where a barrage was constructed across the Zuiderzee to form Ijsselmeer. Both regions are diluted by inflowing rivers and have a mainly lacustrine fauna, they are isolated from the open sea, and the Baltic only experiences negligible tides. Marine species found in the North Sea, Skagerrak and Kattegat have greatly declined in numbers in

the Belt Sea and have disappeared by the entrance to the Baltic. In Ijsselmeer most marine species had disappeared by 1939, seven years after the barrage had been constructed (Beaufort (1953).

Remane (1934) and Segerstråle (1949, 1957) have discussed the general ecological aspects of brackish water communities, using data from the Baltic; Remane presented curves which described the changes in species diversity of the fauna in relation to salinity.

Johannsen (1918) investigated the fauna of Randersfjord and Forsman (1951, 1956) discussed the invertebrate fauna of the Swedish coast, though like most workers they were fundamentally interested in particular taxonomic groups, the former in Mollusca and the latter in Gammarus duebeni. Silfvenius (1906) records the distribution of many species of Trichoptera with respect to salinity along the Finnish coast, and Lindberg (1936, 1948) studied the Baltic distributions of the Hemiptera and Coleoptera. Schlesch (1937) and Jaeckel (1950) both worked on the Mollusca whilst Remmert (1955a, 1955b) surveyed the dipterous fauna in great detail.

In the Netherlands, Redeke (1935) reported the results of an extensive survey of the Zuiderzee before the barrage, and Beaufort (1953) reviewed the changes that occurred in the fauna as Ijsselmeer became progressively more dilute. Van der Velde and Polderman (1974) produced notes on the distribution of Corixidae in relation to salinity in a creek in Zeeland. The continental seas of the U.S.S.R resemble the Baltic, and Zenekevitch (1959) reviews the faunal composition of these.

Ecological investigations of inland saline waters have come mainly from other parts of the world and include waters associated with geological deposits of sodium chloride, waters receiving industrial brine wastes, and mineral lakes where salinity is due to leaching. The last are often highly saline

in arid regions and have heterogeneous ionic compositions.

There are a number of springs and brine-pits associated with the salt deposits in the Cheshire Basin and yet there have been few investigations of their flora and fauna. Burke (1942a) found a filamentous marine alga in a brine spring at Aldersey, near Clutton, and Burke (1942b) recorded Ephydra riparia and Nemotelus notatus Zetterstedt from a brine pit at Lower Wych, near Malpas. The Coleoptera of the area have been studied by Balfour-Browne who found a number of mixohaline species (Garth Foster, pers.comm) and Savage (1971) investigated the corixid fauna of the saline subsidence lakes around Sandbach.

The saline waters associated with the saltfields of Germany have received rather more attention. Lists of species associated with the Westphalian brines have been provided by Schmidt (1913) and Thienemann (1913), and the chironomid fauna in particular has been described by Thienemann (1915, 1954). A similar catalogue of species found associated with the saline waters of Oldesloe, in Holstein, is provided by Thienemann (1925). Schmitz (1959) included details of investigations from both localities when he presented his review of inland saline waters as a contribution towards the classification of brackish waters. In East Germany, Schuster and Mohrig (1970) surveyed the mosquito fauna of the saline waters in the Magdeburg area and recorded a number of species, including Aedes detritus, which are typical of saline habitats.

The ecological effects of brine wastes have been studied in Britain, West Germany and America. Holland (1967a, b) studied the Malacostraca of the Trent and Mersey Canal which receives a saline industrial effluent, and the Bridgewater Canal where the salinity is raised by leaching from saliferous rocks. In West Germany, brine-wastes from potash mines contaminate several rivers and streams. Hirsch (1918) recorded the distributions of a number of freshwater species in relation to salinity in one of these, the River Wipper, and Schmitz (1956), although primarily

interested in the fisheries aspects, discusses changes in the invertebrate fauna of the River Werra. Albrecht (1954) conducted faunal surveys of both rivers and subsequently Ziemann (1968) investigated their diatom floras. In the oil producing states of America the effects of brine wastes from exhausted oil wells have been studied by Biglane and Lafleur (1954) and by Clemens and Jones (1954) who also made laboratory investigations of the toxicity of the wastes to a number of species.

The most extensive study of inland mineral lakes was that conducted by Rawson and Moore (1944) who examined sixty saline lakes of the non-chloride type in Saskatchewan. At high salinities the Diptera predominated; Ephydra spp. Eristalis sp. and Ceratopogonidae were all recorded in hypersaline waters. In Utah, Great Salt Lake, with a salinity of around 203°/oo, has received a great deal of attention (Schwarz 1891, Vorhies 1917, Alleé 1926, Patrick 1936, Woodbury 1948, Zahl 1967). The Ephydridae are the predominant group and several species from this and other saline lakes of the region were described by Aldrich (1912). Thorpe (1931) gives insect records from saline waters in the Californian desert. Beadle (1932, 1943b) studied saline lakes on the African continent where, in both chloride-based and alkaline lakes, he recorded a number of holeuryhaline Diptera, including Ephydra sp., Eristalis sp, and Culicidae.

The faunas of inland saline waters are discussed in general terms by Hesse, Alleé and Schmidt (1937) and salinity tolerances of species which characterise inland saline and hypersaline waters are reviewed by Bayly (1972). Remane (in Remane and Schlieper (1958)) has provided the most comprehensive review of the ecology of brackish waters, and in this he has included a number of inland saline localities. The classification of brackish and inland saline waters was discussed at the Symposium on the Classification of Brackish Waters(Anon,1958)and at the Symposium on Salt and Brackish Inland Waters (Bayly, 1968).

Laboratory investigations of salinity tolerance.

Experimental investigations of the salinity tolerance of various organisms have involved either dose-effect experiments (usually determination of median lethal dose), behavioural experiments, or investigations into osmotic and ionic regulation and water balance.

Gresens (1928) established the lethal dosages of seawater for a number of invertebrates, including Asellus aquaticus L., and demonstrated that tolerance is affected by a number of factors. In particular, he showed that acclimation may increase the ability of an organism to tolerate high salinities, Haage (1968) investigated dose effects of sea water on each stage of development of Phryganea grandis L. and demonstrated that the narrowest range of tolerance is during early ontogeny. Haage (1969) compared P.grandis which he had taken from brackish water habitats with specimens taken from freshwater habitats, and found that the former had a greater tolerance of raised salinities. Furthermore, in alternative chamber experiments, larvae from each source showed a salinity preference for the solution most like their own biotope water. Lagerspetz (1958) conducted simple dose-effect experiments using Asellus aquaticus of freshwater and brackish water origin and demonstrated that animals from a brackish water population in the Baltic have practically lost their ability to tolerate freshwater.

Investigations of the mechanisms of water balance in various invertebrates have been conducted by several workers and although most of these have involved true mixohaline species, a few studies have been made of freshwater invertebrates in saline media.

The alderfly larva, Sialis lutaria L. has been studied by Shaw (1955a, b), and Sutcliffe (1959, 1961b) studied the salt-marsh trichopterous larva, Limnephilus affinis which he later compared

with Limnephilus stigma Curtis and Anabolia nervosa Leach (Sutcliffe 1961 c). L.affinis was able to regulate the electrolyte fraction of the blood and could tolerate rises in blood osmotic pressure of nearly twice those that most freshwater species can tolerate.

A number of mixohaline species have been studied in considerable detail, Aedes detritus being one of the first to attract attention. After the experiments of Wigglesworth (1938)which demonstrated a limited osmoregulatory ability in two freshwater mosquitos, Beadle (1939) demonstrated that A.detritus was capable of powerful hypo-osmotic regulation in concentrated media. Ramsay (1950,1951) showed that active removal of water against an osmotic gradient took place in the rectum.

An even greater ability to concentrate its rectal fluid has been demonstrated in Ephydra riparia (Sutcliffe 1959, 1960a), (the considerable impermeability of the larvae had earlier been demonstrated by Beyer, 1939). Nemenz (1960) investigated another member of the genus, E.cinerea Jones, and found that the larvae are not only extremely impermeable to salts, having a well-defined lipid layer in the cuticle, but that the osmotic pressure of the haemolymph is over three times as great as that of E.riparia.

Sutcliffe (1959, 1960a)also studied osmoregulation in a number of species of chironomid larvae and found a considerable range of abilities to maintain a homoiosmotic state. Neumann (1961) studied Chironomus halophilus Kieffer and Chironomus salinarius Kieffer and found that while the former showed relatively weak powers of hypo-osmotic regulation, C.salinarius had an experimental salinity range of 1-ca 50°/oo. Lauer (1969) investigated Chironomus plumosus L. and Tanypus nubifer Coquillet and found that whereas the latter had some powers of hypo-osmotic regulation, the former probably had not,even though they could apparently survive concentrations of 16°/oos.

The other group of insects to have received attention as regards osmoregulation is the Corixidae, which has mixohaline members

all over the world. Claus (1937) investigated the osmotic behaviour of two freshwater species of corixid, Sigara distincta (Fieber) and S.fossarum (Leach), and that of the mixohaline species S.stagnalis. Whilst the freshwater corixids could not tolerate salinities beyond the isosmotic point, S.stagnalis apparently shows hypo-osmotic regulation between 14-20°/ooS. In British Columbia, Scudder et al (1972) also compared the osmoregulatory ability of a mixohaline species, Cenocorixa bifida (Hung), with a freshwater species, C.expleta (Uhler). They found no indication in either species of sustained hypo-osmotic regulation, nor any ability to produce hyperosmotic urine. The mixohaline species was, however, able to tolerate higher salinities and showed a rather greater ability to regulate haemolymph sodium. In Australia Knowles and Williams (1973) examined two salt-tolerant species, Sigara australis Fieber and Agraptocorixa hirtifrons (Hale) and discovered a very limited ability for hyporegulation.

Tones and Hammer (1975) in Canada, investigated the mixohaline species Trichocorixa verticalis interiores Sailer demonstrating that the insect had well-developed powers of osmoregulation and could hyporegulate in saline water, though not to the same extent as that apparently found by Claus (1937) in S.stagnalis.

One other group of freshwater and brackish water invertebrates whose osmoregulatory ability has been thoroughly investigated is the Gammaridae. Sodium uptake and loss across the body surface and in the urine of Gammarus pulex (L) and G.duebeni from both brackish water and freshwater habitats has been investigated by Shaw and Sutcliffe (Shaw and Sutcliffe, 1961, Sutcliffe 1967a,b; Sutcliffe and Shaw, 1968; Sutcliffe,1971).

Another two brackish water species, G.zaddachi and G.tigrinus, were investigated by Sutcliffe (1968) and the relative osmo-regulatory abilities of the various members of the Gammaridae are discussed in relation to their distribution. Schmitz et al (1967) investigated the dose-effects of solutions of various

ionic composition on G.pulex and G.tigrinus and also on Asellus aquaticus and were able to relate their tolerance to their distribution in the Rivers Werra, Wipper and Ingestre.

A review of physiological features of life in brackish water is provided by Schlieper (in Remane and Schlieper 1958), while Bayly (1972) reviews physiological investigations of organisms living in athalassic and hypersaline waters.

Bayly (1968) discussed the terminology used with reference to inland waters and urged that the term 'brackish' should be avoided in scientific literature when referring to non-marine waters, and suggested that the difficulty should be overcome by using the term 'mixohaline'. This convention is observed in this thesis, the term 'brackish' being used to refer only to diluted sea-water and the term 'mixohaline' being used to include waters of non-marine origin.

CHAPTER II

METHODS

Chemical factors.

Water samples for chemical analysis were collected in 250ml. polythene bottles avoiding the incorporation of air. These samples were returned to the laboratory for quantitative determination of halides, hydrogen ion concentration and conductivity. Samples were kept in the dark and analysed immediately on return to the laboratory. When this was impossible, samples were retained under refrigeration.

Chlorides and the other halides were determined by Mohr's titration with silver nitrate solution using potassium chromate as indicator. The natural brine of the Cheshire basin contains chlorides and bromides in the ratio of 200 : 1, iodine is negligible. Halide determinations made by the Mohr titration were therefore considered to be entirely attributable to chlorides. An assay of Cheshire brine taken at Holford was made by Sherlock (1921) and is presented in table 1. This indicates that a factor of 1.68 can be applied to chlorinity values to give an approximate value for total salinity. Where these estimates are supplied in the text they are given in parentheses.

Sodium and potassium were measured by use of a flame spectrometer (Evans Electroselenium Ltd).

Before August 1972 calcium was determined by titration with EDTA (ethylenediamene-tetra acetic acid) using Murexide (ammonium purpurate) as indicator. Total hardness (calcium and magnesium) was determined by titration with EDTA using Solochrome blue-black RR (Erichrome black T) as indicator. Magnesium values were derived from the difference between the above determinations.

After August 1972, calcium and total hardness were determined using an Atomic Absorption Spectrophotometer (Evans Electroselenium Ltd. model 240).

Table 1. Composition of brine taken near Holford Hall, (from Sherlock, 1921).

NaCl	305.78	g l^{-1}
MgCl	0.8	g l^{-1}
$CaSO_4$	5.03	g l^{-1}
$MgSO_4$	0.9	g l^{-1}
Ca (dissolved)	0.04	g l^{-1}
Total salinity	312.55	g l^{-1}
Chlorides	185.98	g l^{-1}

Ratio of total salinity to chlorides = 1.68 : 1

Hydrogen ion concentration was determined in the laboratory using a Pye pH meter (model 292) calibrated to pH7 using EIL buffer powders.

Oxygen saturation was measured in the field using a Mackereth Mk.II oxygen probe. (The Lakes Inst.Co., Windermere).

Conductivity was determined using an Electrolytic Conductivity Measuring Set, (Model MC-1 Mk.V, Electronic Switchgear).

Throughout the text SI units have been used except for concentrations of biotope water. Frequently the ionic composition of the water was unknown and this made the use of molar units impossible. Historically the salinity of biotope water has been expressed as ppm or $^{o}/oo$, and to maintain continuity with previous work, the units used in this study are mg l^{-1} which is numerically equivalent to ppm, and for extreme concentrations, the unit parts per thousand by weight ($^{o}/oo$) has been preserved. The suffix Cl is used to indicate that the figure given for salinity is for the chloride component only, and the suffix S is used where the value given is that for total salinity. Where it has been necessary to calculate sea water equivalents in terms of chlorides or total salinity, the tables of Barnes (1954) have been consulted. As a general rule, full strength Atlantic sea water has a total salinity of $34.5^{o}/oo$ and a chloride content of $19^{o}/oo$. The composition of sea water is given in a condensed form by Macan (1963, p207).

Physical factors.

Water temperature was measured in the field with a mercury thermometer, range $-10^{o}C$ to $110^{o}C$ Where details of rainfall, air temperature and occurrence of anticyclonic conditions were required, they were taken from the records of the Keele Meteorological Station.

The fauna.

Faunal samples were obtained by different techniques at the various habitats and methods of sampling are discussed in detail in the appropriate chapters.

The samples were taken to the laboratory in 4 litre buckets where they were sieved using a 4 mesh per cm and a 16 mesh per cm sieve. Initially macroinvertebrates were sorted from the residual material by hand. Any weed and organic matter was teased apart carefully. Particular attention was paid to the Mollusca and Trichoptera at this stage. After hand sorting, the sample was flooded with a solution of magnesium sulphate in 2½% formaldehyde with a specific gravity of 1.1 to 1.2 to float out any remaining organisms. The formaldehyde acted as an irritant to encourage emergence of chironomids and oligochaetes from their tubes and from crevices.

Specimens were identified alive where necessary, or preserved for determination at a later date. The preservatives used were those recommended by the appropriate authorities for the groups. Where such information was not available 70% alcohol plus 5% glycerine was used. Where available, keys for identification published in the Freshwater Biological Association Scientific Publications series were used. For other groups the following works were consulted: Gardner,1954 (Odonata); Hickin,1967 (Trichoptera); Kimmins,1956 and Hiley, 1976 (Limnephilidae); Joy,1932 (Coleoptera); Oldroyd,1949 and Coe, Freeman and Mattingly, 1950 (Diptera); Chernovskii,1949 and Bryce and Hobart, 1972 (Chironomidae), Chu, 1949 (immature insects). For general information two texts were used, Ward and Whipple (1918) and Macan (1959).

CHAPTER III

THE EFFECTS OF BRINE ON THE ECOLOGY OF A FRESHWATER STREAM.

INTRODUCTION

Only a few detailed studies have been made of the invertebrate faunas of running waters contaminated by brine, though fisheries investigations have been more frequent in habitats of this type in both West Germany and in the United States of America.

In West Germany saline effluents from the potassium industry of North Rhine - Westphalia are discharged into the River Werra and thence into the River Weser and also into the Wipper and Innerste streams. Hirsch (1918) investigated the biological effects of the elevated salt concentrations in the Wipper and Albrecht 1954 made a detailed study of the invertebrate fauna of not only the Wipper but also the Werra. Using toxity experiments, Schmitz et al (1967) compared the salt tolerance of Gammarus pulex, G.tigrinus and Asellus aquaticus with their known distribution (Schmitz 1956) in the Werra, Weser and Innerste streams. Schmitz (1959) later reviewed the results of the surveys in this region of W.Germany and from other inland saline waters of Europe, as a contribution towards producing a classification of saline waters.

In America investigations of river faunas affected by brine discharges have been carried out in the oil producing states, where exhausted oil wells ultimately produce salt water (principally sodium chloride), and discharges into river systems have occurred on a large scale. Clemens and Jones (1954) tested the toxicity of brine from oil wells to ten species of fish and ten species of invertebrates, including Baëtis rhodani Pictet & Tubifex sp, in laboratory conditions, and produced figures for Median Lethal Dose of the brine for each species. Biglane and Lafleur (1954) made an ecological survey of an 80km stretch of a Louisiana river which received oil field brines and had a highly variable salinity. They found that even salt tolerant fish showed a

preference for freshwater, a phenomenon which they attributed to an impoverished fauna in the saline reaches where the only invertebrates to be found consistently were two undetermined midge larvae.

The investigation presented here is a detailed survey of the invertebrate fauna of a Cheshire stream receiving run-off from controlled pumping of brine on the largest brinefield in Britain.

Description of the site.

Crow Brook flows across the Holford brinefield from east to west for just over 2 km of its course. (Fig.2). The brinefield is an area of pastoral land of several hundred hectares, situated 5km ENE of Northwich. Brine is extracted from controlled cavities in the Lower Keuper Saliferous Beds where boreholes are arranged on a regular grid pattern, on average some 180m apart. Concentrated brine is continually discharged from the boreholes and is precipitated from projecting pipes into the centre of Crow Brook (plate 1). The input of brine varies from trickles to occasional massive discharges. Eleven sampling stations were selected along the brook, the first upstream of all brine discharges and the other ten progressively downstream at intervals of between 150m and 400m. Each sampling point was downstream from a brine outflow at a sufficient distance to allow complete mixing of the brine with the stream water.

The stream itself (Plate 2) is approximately 3m wide with an average depth of approximately 0.3m at normal flows. The deep banks accommodate water 1.2m deep in times of spate. The substratum is sandy mud containing pebbles of all sizes. All sampling stations were situated at riffles.

The course of the stream is sparsely lined with a variety of trees and bushes, chiefly alder (Alnus glutinosa (L)), hawthorn, (Crataegus monogyna Jacquin) and willow (Salix alba L).

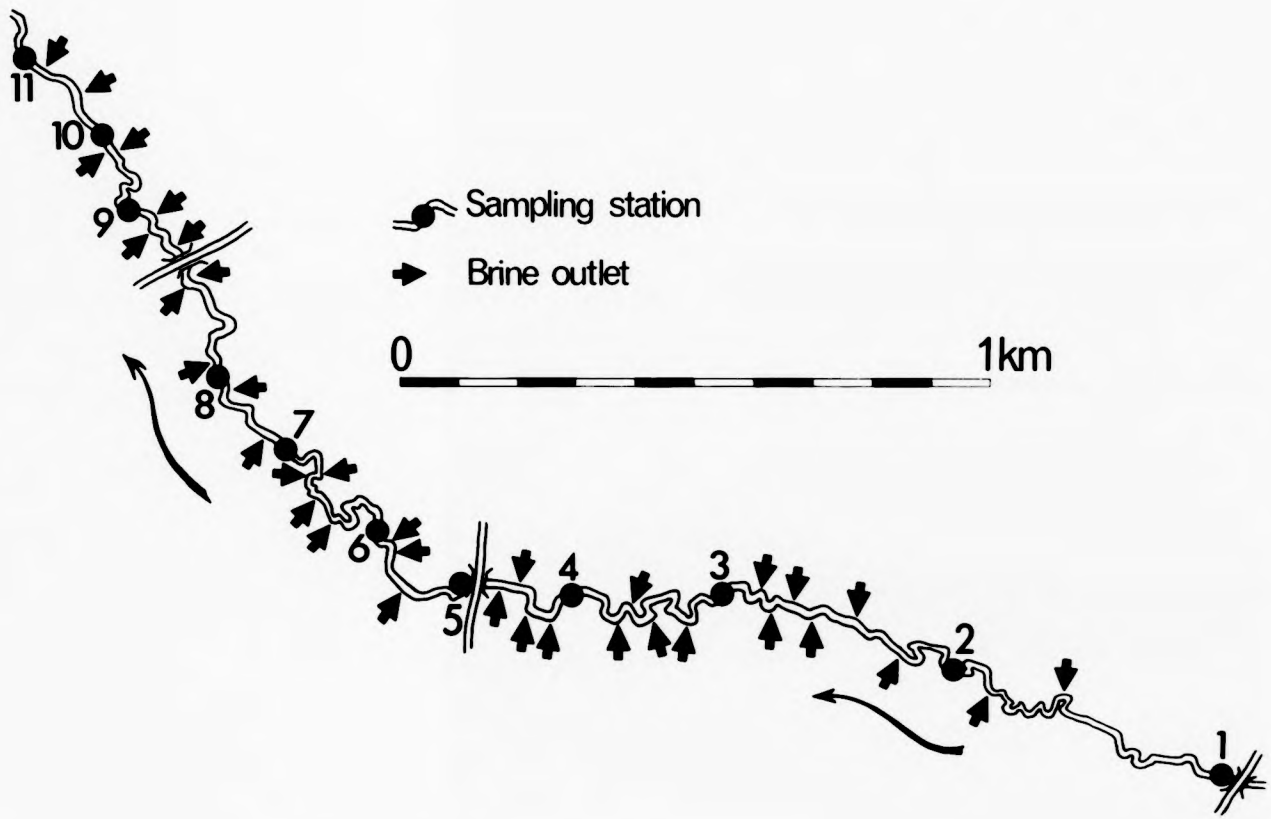


Fig. 2. Map of Crow Brook showing sampling stations and brine outlets.

Plate 1. Discharge of concentrated brine into Crow Brook.

Plate 2. Crow Brook, sampling station number 8.

METHODS

Sampling programme.

Water samples for chemical analysis and faunal samples were taken at approximate monthly intervals on eleven occasions between 12th September 1972 and 13th August 1973. The stream was visited on 10th November 1972 but as it was in spate, it was only possible to take water samples. The stream was also in spate throughout July 1973 and again only water samples were taken that month.

Water Quality.

(a) Chemical factors. Salinity, conductivity and pH were measured for all stations every time the brook was visited. The concentration of neat brine and the diluting capacity of the stream were assessed on 29.11.72. Calcium and magnesium levels were measured at the freshwater stations (1 and 2) on 20th December 1972. Analysis of the brine for hardness was provided by I.C.I. The oxygen levels were determined at noon at every station on both 19th January, 1973, and 11th June, 1973, and at midnight on 28th June 1973.

(b) Physical factors. The temperature of the stream water was taken at approximately midday at each station on every visit. Current velocity was measured at station 6 on 12th September 1972 using a Kent Type 265 'Miniflo' current velocity meter. The rainfall record was obtained from the Keele Meteorological station for the week preceeding each visit to the stream.

Biological investigations.

(a) The flora.

Macrophytes were scarce in the stream, probably as a result of shading, and quantitative assessment of abundance was only made for Fontinalis antipyretica L. The alga Enteromorpha

intestinalis L. was abundant in the saline reaches of the stream and on 17th April and 11th June all filaments of the alga and all strands of F.antipyretica within a one ft^{2*} Surber frame were stripped from the pebbles at each station and returned to the laboratory for dry weight determinations.

(b) The fauna.

Invertebrates were collected with a Surber bottom sampler, one sample being taken from the stony riffles of every station. Organisms were transported, extracted and preserved as described in Chapter II. All specimens were identified as precisely as available keys permitted and the numbers recorded.

RESULTS

Water quality. (a) Chemical factors.

Salinity parameters for each station are recorded in table 2.

Salinity. Fig.3 shows the mean salinity of each station of Crow Brook over one year and a progressive increase in salinity along the stream is apparent. A high variability is indicated by the large standard errors, the more saline stations being generally more variable.

The variability in salinity was seasonal rather than random (Fig.4) and the mean salinity of the whole stream on each sampling occasion showed an inverse relationship to rainfall (Fig.5). The least extreme conditions were therefore initially seen in the autumn of 1972 when salinities were moderate, and later in November and December when salinities were low. Higher salinities occurred through the spring and early summer reaching a maximum in June 1973. An unexpectedly low salinity was recorded in July when the stream was in spate following a week of abnormal rainfall.

*one ft^2 is treated as equivalent to $0.1m^2$ for the purposes of this study.

Table 2. Salinity ($mgl^{-1}Cl$) along Crow Brook.

Station number	1	2	3	4	5	6	7	8	9	10	11
Maximum salinity	220	350	3940	4000	5280	5200	10800	11600	7900	8300	15800
Mean salinity	67	120	1570	1600	2150	2200	3140	3560	3340	3800	4190
Minimum salinity	31	38	60	190	230	240	280	300	330	340	350
Standard deviation	60	90	1080	1080	1470	1480	2880	3140	2320	2770	4470
Coefficient of normal instability. $(\frac{\text{s.d.}}{\text{mean Cl}})$	0.89	0.77	0.69	0.68	0.68	0.67	0.92	0.88	0.69	0.73	0.94
Coefficient of absolute instability. $(\frac{\text{range}}{\text{mean Cl.}})$	2.82	2.63	2.40	2.38	2.35	2.26	3.36	3.12	2.27	2.10	3.69

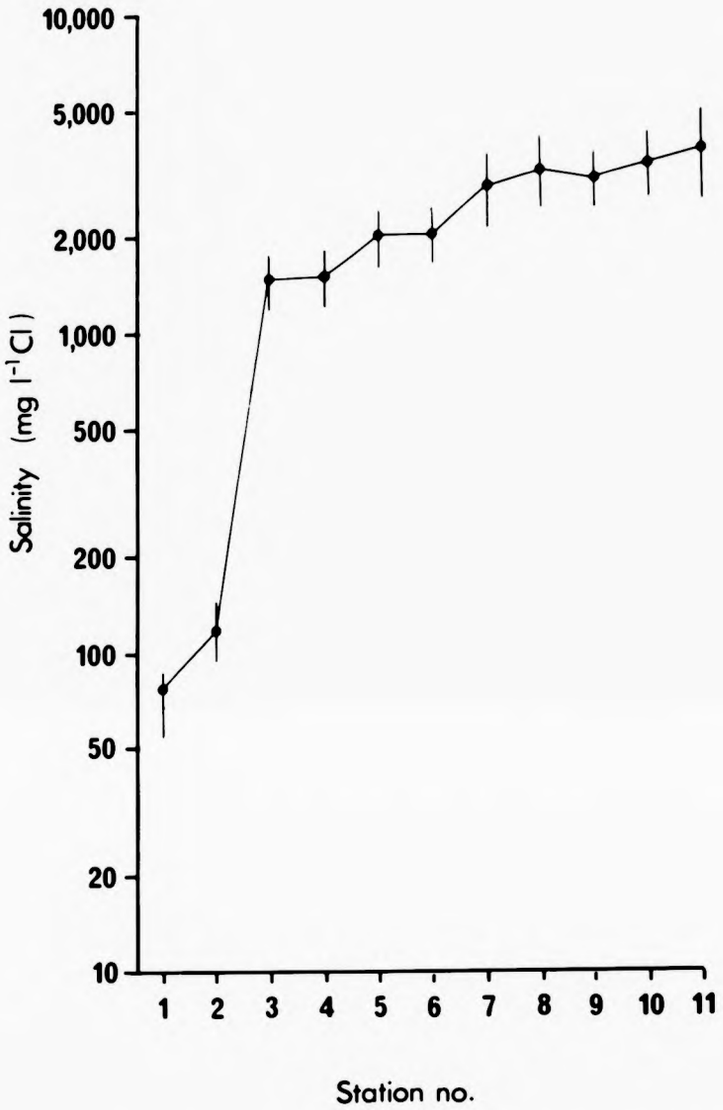


Fig. 3 Mean salinity (±standard error) at stations along Crow Brook

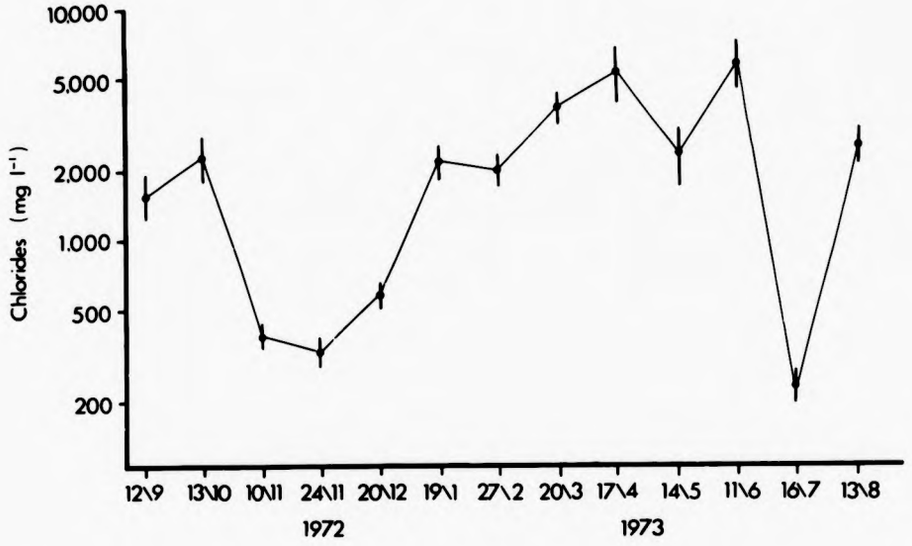


Fig. 4 Seasonal variation of mean salinity of Crow Brook during the period of study

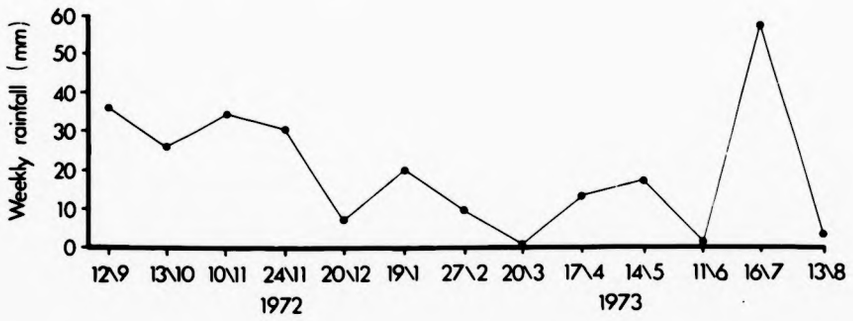


Fig. 5 Precipitation for weeks during which samples were taken

Shock increases in salinity due to borehole pressure releases were difficult to record as these were unpredictable. It was possible, however, to monitor one such discharge on 29.11.72 when concentrated brine of 195 °/oo Cl was released just above station 4. A comparison between salinity levels recorded on 24.11.72 and 29.11.72 is made in table 3.

Table 3. Increases in salinity resulting from a borehole pressure release upstream from station 4 on 29th November 1972.

Station No.	Salinity (mgl^{-1} Cl)		
	24.11.72	29.11.72	increase due to discharge.
3	60	360	300
4	300	1850	1550
6	400	1960	1560
10	490	2250	1760

A salinity increase of 1,550 mgl^{-1} Cl occurred at station 4 between the two visits, although the concurrent increase in salinity at station 3 indicates that a general background increase of 300 mg l^{-1} Cl contributed to this. An increase in salinity of 1,250 mg l^{-1} Cl is therefore attributable to brine discharged during the release of pressure of the borehole. The diluting capacity of the stream was therefore only 150, and the severity of the discharge was compounded downstream, (viz. salinity at stations 6 and 10), probably due to inadequate dilution and by additional minor discharges. Salinity in the stream is therefore probably intermittently more severe than found at the routine surveys.

Conductivity. The regression of conductivity (y) on chlorinity (x) is described by $y = 0.0024x + 1.05$ for salinities above 1000 mg l^{-1} Cl (correlation coefficient, $r = 0.988$) and by $y = 0.03x + 3.65$ for salinities below 1,000 mg l^{-1} Cl ($r = 0.969$). The lower gradient of the regression line for the less saline

waters was due to the greater relative contribution of other ions to the total conductivity.

Hydrogen ion concentration. The pH of Crow Brook did not vary greatly from neutral. The mean pH at station 1 was 7.3 with a standard deviation of 0.34 over the year, whereas the mean pH at station 11 was 7.4 with a standard deviation of 0.32. A t test showed that these differences were not significant, (Appendix 1) so any effect of the brine on the pH of the stream could be discounted.

Hardness. Samples from the freshwater stretch of the stream were analysed for hardness on two occasions. The baseline level of calcium in the stream was 167 mg l^{-1} and the total hardness was 243 mg l^{-1}. The degree of hardness contributed by the brine was calculated from analyses of the brine provided by I.C.I which gave its composition as 0.72% calcium and 0.12% magnesium. The maximum salinity recorded in the stream was 15,800 mg l^{-1} Cl which would have increased total hardness by only 139 mg l^{-1}.

Dissolved oxygen. Concentrations of dissolved oxygen in the stream in January and June 1973 are given in table 4. Levels were high in January suggesting that biological oxygen demand (BOD) was low at this time of the year. Although concentrations were consistently lower at the more saline stations during the day, the trend was reversed at night and probably results from a higher BOD at the saline stations rather than the reduction in solubility of oxygen with increased salinity. The midnight visit on 28th June showed a further decrease in oxygen levels and this was almost certainly due to cessation of photosynthesis.

(b) Physical factors.

Water temperature. The influx of brine to the stream apparently did not affect the water temperature as the variation between stations was both random and small (less than $1^{o}C$) on any one sampling occasion. Seasonal variation in water temperature is shown in figure 6 and is sinusoidal having a minimum of $3^{o}C$ in January and February and a maximum of $18^{o}C$ in June.

Table 4. Concentration of dissolved oxygen in Crow Brook.

Date	Time	Approximate temperature (°C)	Dissolved oxygen at each station. mg l^{-1} (% saturation)										
			1	2	3	4	5	6	7	8	9	10	11
19.1.73	midday	1.8 - 3.1	10.2 (79)	10.2 (78)	10.3 (78)	10.3 (78)	10.2 (77)	10.1 (76)	10.4 (77)	9.9 (74)	9.3 (70)	9.1 (68)	9.2 (68)
11.6.73	midday	19.5 - 24.0	6.8 (74)	5.7 (64)	5.7 (63)	4.6 (53)	3.8 (46)	3.8 (46)	4.0 (48)	4.4 (52)	4.8 (58)	4.6 (55)	4.8 (57)
28.6.73	midnight	16.3 - 16.4	2.9 (30)	4.0 (41)	2.9 (30)	2.9 (30)	3.5 (36)	3.8 (39)	4.0 (42)	4.2 (44)	4.3 (45)	4.3 (45)	4.6 (48)

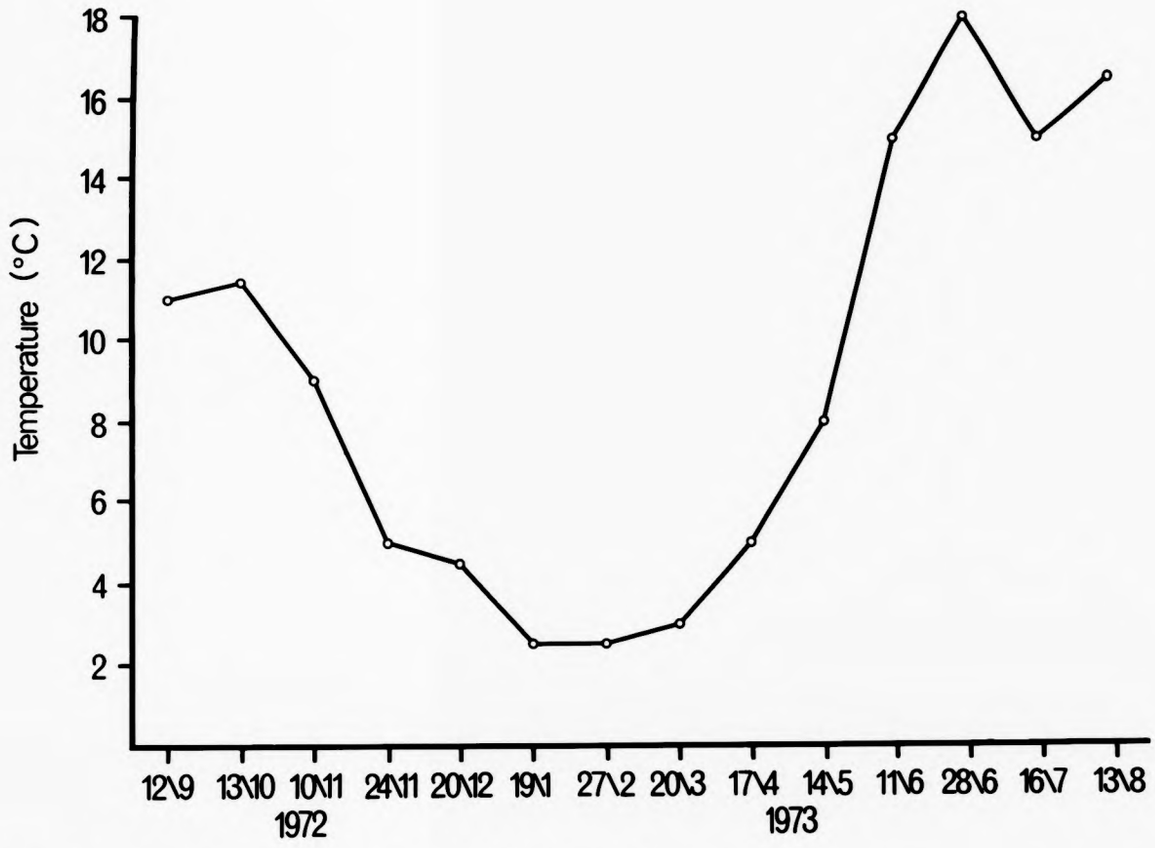


Fig. 6 Midday water temperatures of Crow Brook

Current speed. Rate of flow was measured on 12th September 1972 when it was probably a little above average. The current speed varied between 144 cm sec^{-1} in the faster parts of the riffles and 68 cm sec^{-1} in the slower parts.

Biological components.

(a) The flora.

Although aquatic macrophytes were sparse, Callitriche sp. was present at stations 1-4 and Fontinalis antipyretica was present at stations 1-7. The abundance of both species was reduced with increasing distance downstream. Quantitative samples taken on 17:4:73 and 11:6:73 gave a mean dry weight of 20g m^{-2} F.antipyretica at the riffles of stations 1-5 whereas the only downstream record was 0.45gm^{-2} dry wt at station 7. Enteromorpha intestinalis was sampled on the same two occasions, and although it was absent from the two freshwater stations, it reached a maximum density of 22.4 gm^{-2} in June at station 7 (figure 7). A decline was apparent further downstream and this contrasted with the progressive increase in abundance of an unidentified filamentous alga from station 7 to station 11 in the summer of 1973. The abundance of E.intestinalis showed a strong inverse relationship to levels of dissolved oxygen in the stream and oxygen depletion may be caused by both a high oxygen demand of this alga at night and by decomposition of trapped detritus in the fronds.

(b) The Fauna.

Difficulties were encountered in relating species distribution and species diversity to absolute salinity in an environment of high variability. At each sampling point two parameters had to be considered as causing osmotic stress, firstly the salinity per se and secondly the variability of the salinity. Den Hartog (1964) proposed the use of two coefficients of variability, "normal instability" which is defined as the average yearly chlorinity oscillation divided by the average chlorinity ($\frac{\sigma_A}{S}$) and "absolute instability" which is defined as

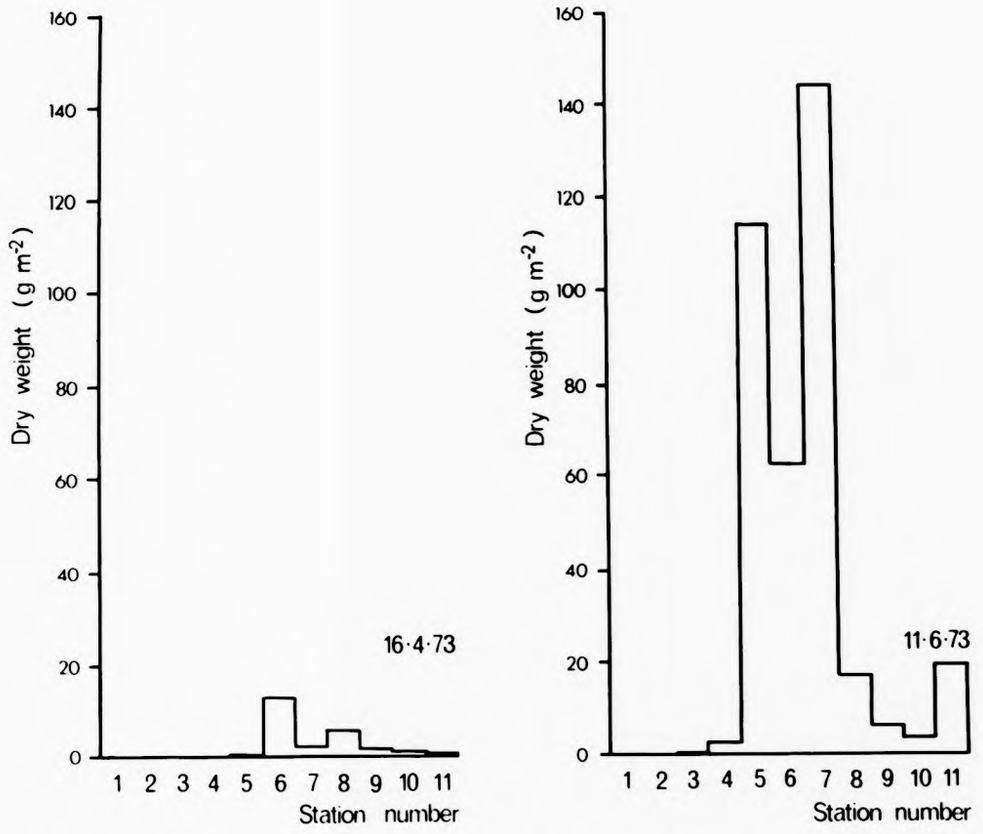


Fig. 7 Abundance of Enteromorpha intestinalis along Crow Brook on 16·4·73 & 11·6·73

the maximum yearly chlorinity oscillation divided by the average chlorinity ($\frac{\text{range}}{\bar{s}}$). However, as salinities increased along Crow Brook the general trend was for variability to increase (table 2), the coefficients of instability thus remained constant. Therefore, although the stream can be described as having a high instability along its length, the coefficients do not assist in describing the severity of the environment at successive sampling points. Species distributions and species diversity are therefore presented with reference to station number as the environment generally became both more saline and more variable with distance downstream.

Species present and their distribution. A total of sixty-five species of macroinvertebrates was recorded in the stream and these have been allocated to categories of degree of tolerance of salinity in accordance with the terminology of Remane and Schlieper (1958) and are presented in table 5. Many of the species were sufficiently abundant to allow their distributions to be plotted against stations of increasing salinity (fig.8a-f). Quantitative assessments of Tubificidae, Naidae and Enchytraeidae could not be made owing to their large numbers, although a decrease in density of Tubificidae was apparent in the lower reaches of the stream.

Composition of the fauna at each station. Numbers of each species found at each station are collated in table 6. The total numbers of invertebrates recorded at each station and the percentage contribution of various major taxa are summarised in fig.9, (Tubificidae,Naidae and Enchytraidae are excluded). The rest of the fauna was divided into five categories. Category I included the soft-bodied invertebrates from the lower phyla i.e, the Lumbricidae, Hirudinea and Mollusca. The second category consisted principally of the Crustacea, though Collembola, Heteroptera and Coleoptera were included in this category and separated from the other insect groups as they were considered to have a degree of independence from salinity by virtue of their

 Text continues on P.47.

Table 5. List of species found in Crow Brook.

Group I. Stenohaline and euryhaline limnobionts of the first degree, ie, strictly freshwater species and species extending from freshwater to waters of low salinity.

Class: Hirudinea.		
	Rhynchobdellida: (Glossiphoniidae)	Glossiphonia complanata L.
		Helobdella stagnalis L.
	Pharyngobdellida: (Erpobdellidae)	Erpobdella octoculata L.
Class: Insecta.		
	Ephemeroptera: (Baëtidae)	Baëtis rhodani Pictet
	Trichoptera: (Hydropsychidae)	Hydropsyche pellucidula (Curtis)
		Hydropsyche angustipennis (Curtis)
	Coleoptera: (Elminthidae)	Elmis aenea Müeller
	Diptera: (Chironomidae)	Potthastia longimanus Kieffer
		Synorthocladius semivirens Kieffer
		Pentaneura sp.

Group II. Euryhaline limnobionts of the second degree, ie, species extending from freshwater to waters of moderate salinity.

Class: Oligochaeta.		
	Opisthopora: (Lumbricidae)	Eiseniella tetraedra Sav.
Class: Crustacea		
	Amphipoda: (Gammaridae)	Gammarus pulex L.
Class: Insecta.		
	Trichoptera: (Limnephilidae)	Potamophylax sp.
	Diptera: (Tipulidae)	Dicranota sp.
	(Chironomidae)	Polypedilum sp. "laetum" group.

	Polypedilum sp. "nubeculosum" group.
	Polypedilum sp. "convictum" group.
	Brillia modesta Meigen
	Eukiefferiella claripennis Lundbeck
(Empididae)	Hemerodroma sp.

Group III. Euryhaline limnobionts of the third degree, ie, species extending from freshwater to waters of high salinity.

Class: Oligochaeta.

Plesiopora (Tubificidae)	Tubifex tubifex Müller
	Limnodrilus hoffmeisteri Claparède
Prosopora (Lumbriculidae)	Lumbriculus variegatus Müller

Class: Insecta.

Diptera (Chironomidae)	Rheocricotopus effusus Walker
	Diplocladius cultriger Kieffer
	Euorthocladius thienemani Kieffer
	Eukiefferiella sp.
(Simulidae)	Simulium ornatum Meigen

Group IV. Halophye organisms, i.e, freshwater species which attain maximum abundance in moderate salinities.

Class: Gastropoda.

Mesogastropoda (Hydrobiidae)	Potamopyrgus jenkinsi Smith

Class: Crustacea.

Isopoda	Asellus aquaticus L.

Class: Insecta.

Trichoptera (Hydroptilidae)	Hydroptila tineoides Dalman.
Diptera (Chironomidae)	Macropelopia sp.
	Apsectrotanypus trifascipennis Zetterstedt
	Psectrotanypus varia Fabricius
	Microtendipes sp.
	Prodiamesa olivacea Meigen
	Metriocnemus atratulus Zetterstedt
	Chironomus riparius Meigen
(Muscidae)	Limnophora sp.

Group V. Genuine brackish water species, i.e, species apparently confined to briny water.

Class: Crustacea.

Amphipoda (Gammaridae)	Gammarus duebeni Lillj.

Group VI. Holeuryhaline species, i.e, those species whose distribution ranges from freshwater to waters of extreme salinity.

Class: Oligochaeta.

Plesiopora (Naididae)	Nais elinguis Müller
(Enchytraeidae)	Lumbricillus reynoldsoni Backlund

Group VII. Species independent of the aquatic environment.

Class: Arachnida.

Acarina (Hydracarina)	Unidentified.

Class: Insecta.

Collembola	Unidentified.

Group VIII. Species recorded occasionally.

Class: Turbellaria.		
	Tricladida (Planariidae)	Polycelis tenuis Ijima
Class: Oligochaeta.		
	Plesiopora (Tubificidae)	Limnodrilus udekemianus Claparède
Class: Gastropoda.		
	Basommatophora (Limnaeidae)	Lymnaea (radix) pereger Müller
	(Ancylidae)	Ancylastrum fluviatile Müller
Class: Pelecypoda.		
	Protobranchia (Sphaeridae)	Sphaerium sp.
Class: Crustacea.		
	Ostracoda	Unidentified.
Class: Insecta.		
	Plecoptera (Nemouriidae)	Nemoura cinerea Retzius
	Hemiptera (Mesoveliidae)	Mesovelia furcata Mulsant & Ray
	Coleoptera (Hydrophilidae)	Cercyon sp.
	Diptera (Culicidae)	Aedes detritus Haliday
	(Tipulidae)	unidentified.
	(Chironomidae)	Procladius sagittalis Kieffer
		Chironomus anthracinus Zetterstedt
		Chironomus plumosus L.
		Einfeldia sp.
		Potthastia gaedi Meigen
		Cricotopus sylvestris Fabricius

	Eukiefferiella graeci Edwards
(Ceratopogonidae)	unidentified.
(Syrphidae)	Tubifera sp.
	Pericoma sp.

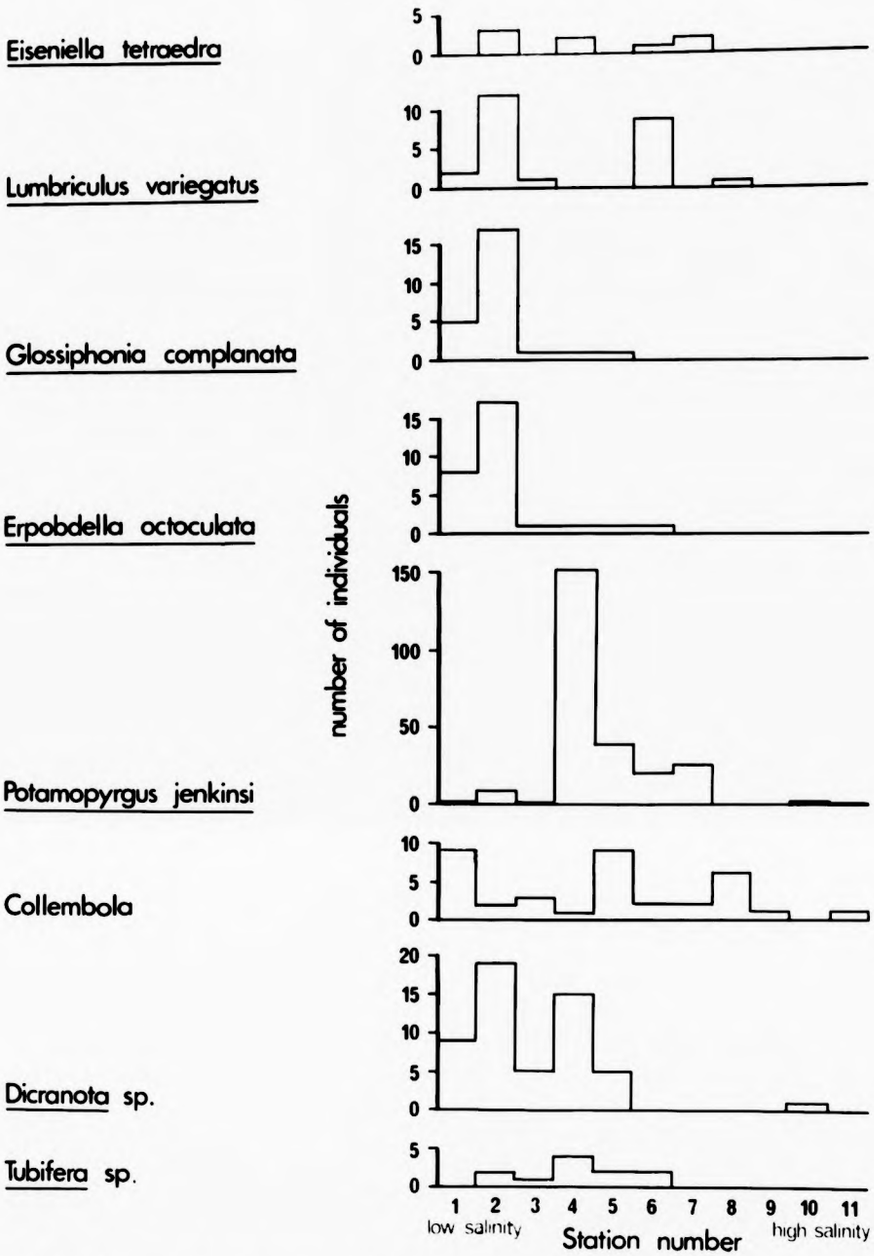


Fig 8a Cumulative frequency of invertebrates taken from stations along Crow Brook between August 1972 and August 1973

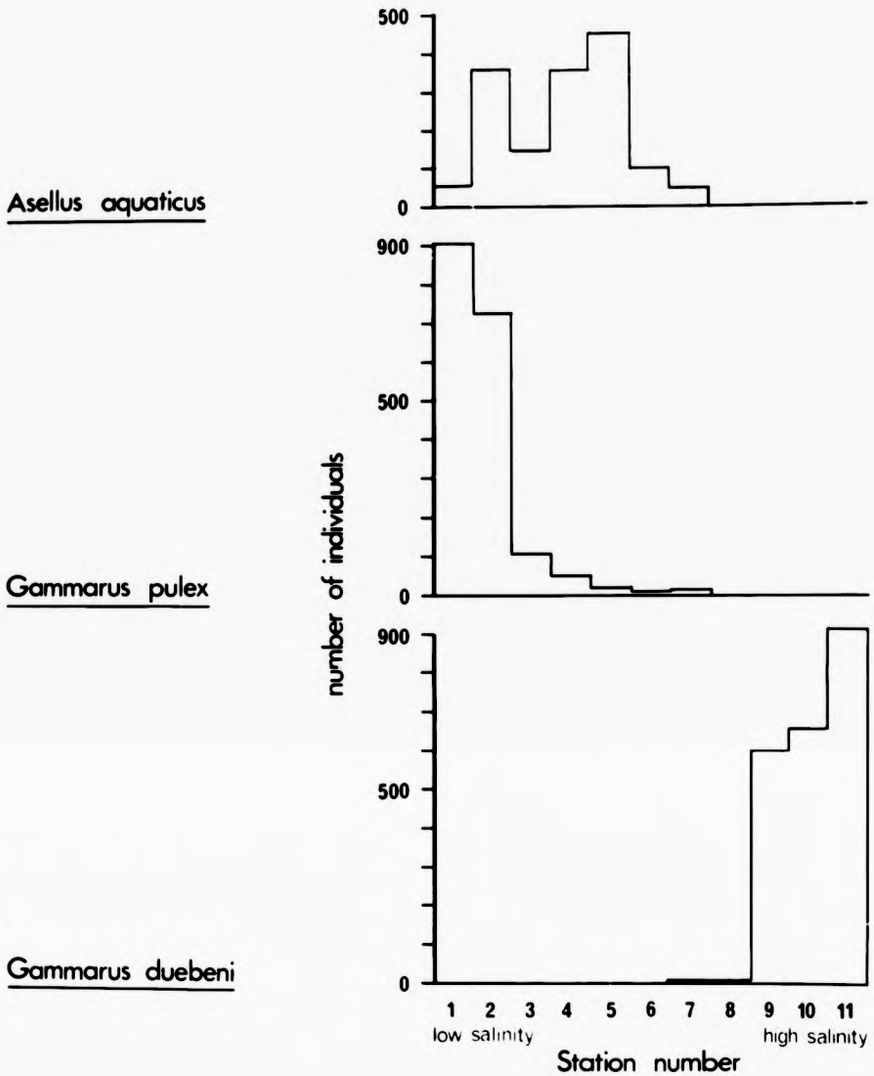
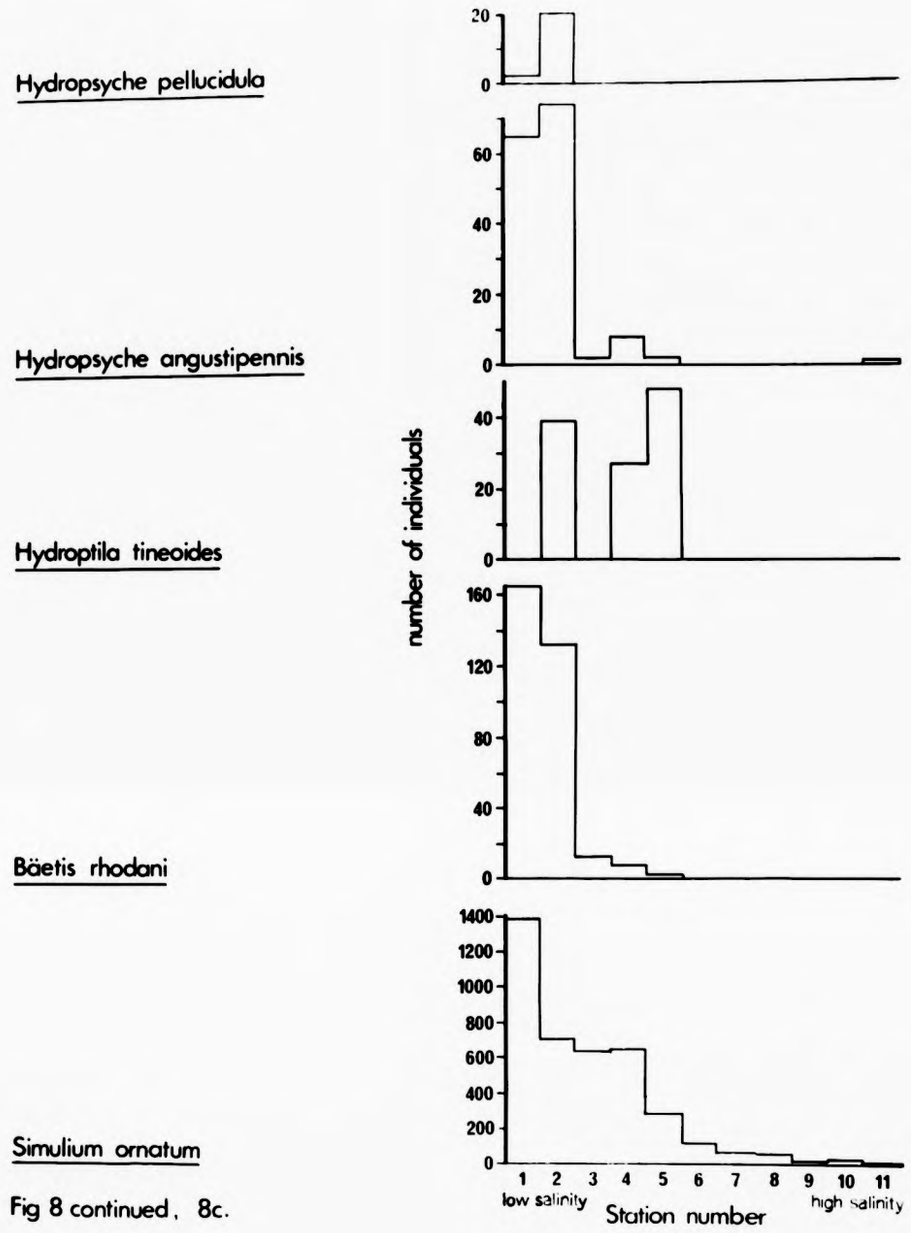


Fig 8 continued. 8b Crustacea.



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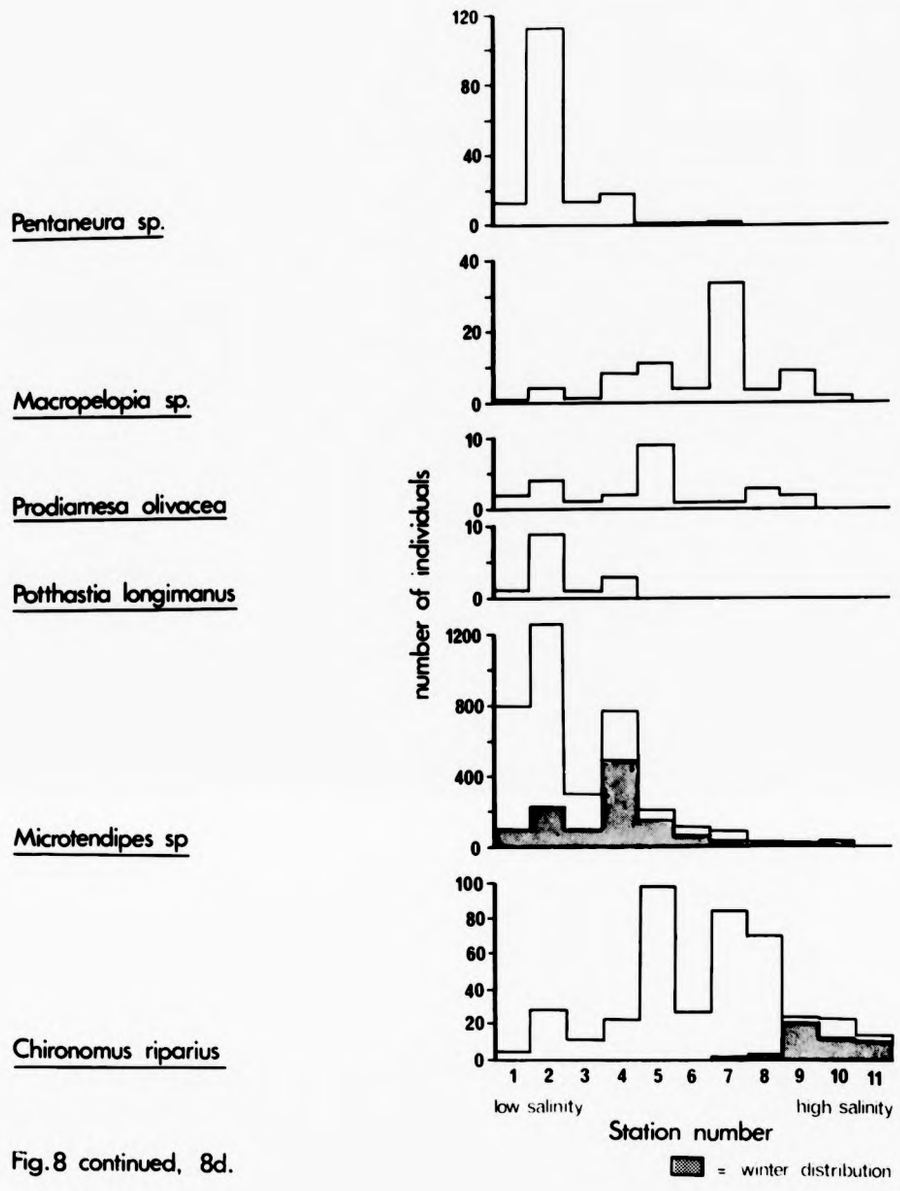


Fig. 8 continued, 8d.

Polypedilum spp.

Diplocladius cultriger

Metriocnemus atratulus

Brillia modesta

Eukiefferiella sp.

Eukiefferiella claripennis

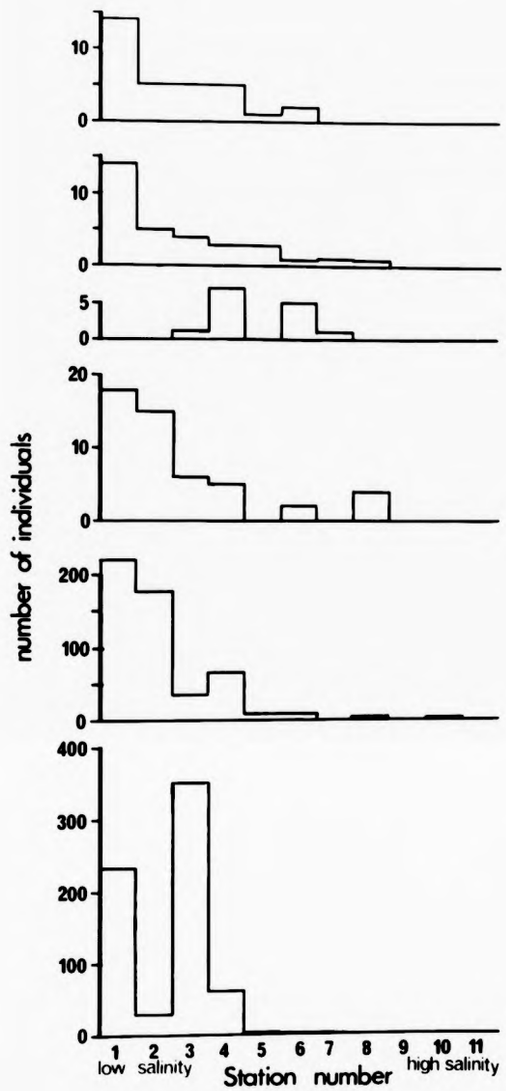


Fig. 8 continued, 8e.

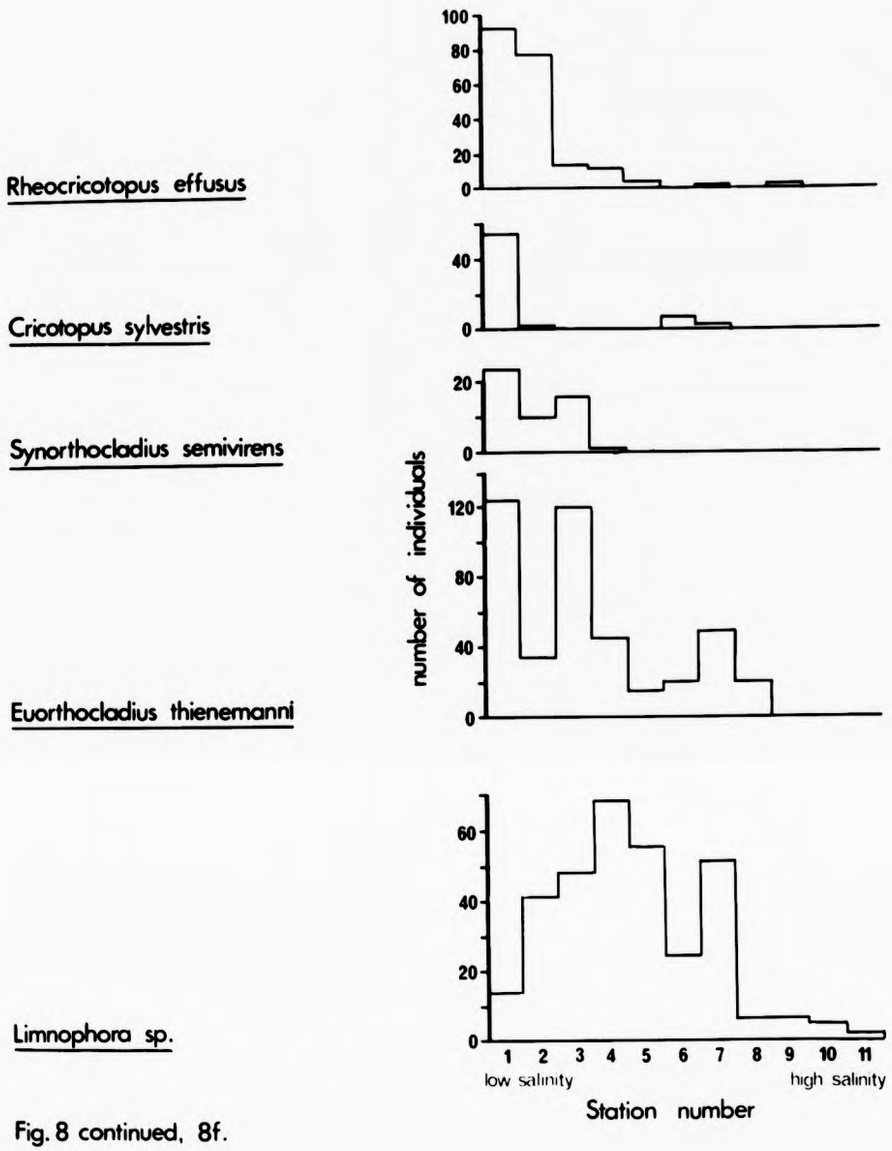


Fig. 8 continued, 8f.

Table 6. Total numbers of each species taken at each station along Crow Brook during the survey.

	STATION NUMBER										
	1	2	3	4	5	6	7	8	9	10	11
CATEGORY 1.											
Immature Tubificidae	x	x	x	x	x	x	x	x	x	x	x
Tubifex tubifex	x	x	x	x		x			x		
Limnodrilus udekemianus	x	x						x			
L.hoffmeisteri	x	x	x	x	x	x		x	x		
Nais elinguis	x	x	x	x	x	x	x	x	x	x	x
Enchytraeidae	x	x	x	x	x	x	x	x	x	x	x
Polycelis tenuis		2									
Eiseniella tetraedra		3		2		1	2				
Lumbriculus variegatus	2	12	1			9		1			
Glossiphonia complanata	5	17	1	1	1						
Helobdella stagnalis		1									
Erpobdella octoculata	8	17	1	1	1	1					
Potamopyrgus jenkinsi	1	9		152	38	21	26			2	1
Lymnaea pereger					1						
Ancylastrum fluviatile	1					1					
Sphaerium sp.		1									
Total (excluding Naiidae, Tubificidae, and Enchytraeidae.)	18	62	3	157	41	33	29	1		2	1
CATEGORY 2.											
Ostracoda (unidentified)	1			1							
Asellus aquaticus	56	363	146	361	448	103	53	1	1	1	
Gammarus pulex	904	732	113	51	20	10	15				
Gammarus duebeni					1	2	13	13	602	661	911
Hydracarina (unidentified)	4		1			5	1				
Collembola (unidentified)	8	2	3	1	8	2	2	6	1		1
Mesovelia furcata		1									
Cercyon sp.	2	1									
Elmis aenea	1	5									
Total	976	1103	263	414	479	122	84	20	604	662	912
CATEGORY 3.											
Nemoura cinerea		2		1				1			
Baëtis rhodani	165	132	13	8	3	1					
Hydropsyche pellucidula	2	12									1
H. angustipennis	65	43	2	7	2	1					
Potamophylax sp.	5	4			1			2			
Hydroptila tineoides		39			27	48					
Total	237	232	15		33	50		3			

x = species present

continued overleaf

Table 6 continued.

	STATION NUMBER										
CATEGORY 4.	1	2	3	4	5	6	7	8	9	10	11
Chironomus riparius	5	28	11	23	98	27	84	70	23	23	14
C. anthracinus								1	1		
C. plumosus							4				
Einfeldia sp.	1										
Polypedilum laetum	3										
P. nubeculosum	10		1	2		2					
P. convictum	1	5	2	3	1						
Microtendipes sp.	805	1251	303	774	209	114	93	26	18	29	2
Pentaneura sp.	13	113	14	19	1	1	2				
Macropelopia sp.	1	1	1	8	11	4	34	3	9	2	
Apsectrotanypus trifascipennis				2	1		7	1			1
Psectrotanypus varia		2									
Procladius sagittalis		2									
Total	839	2799	332	831	322	148	224	101	51	54	17
CATEGORY 5.											
Prodiamesa olivacea	2	10	1	2	9	1	1	3	2		
Diamesa gaedi										1	
Potthastia longimanus	1	9	1	3							
Brillia modesta	18	15	6	5		2		4			
Metriocnemus atratulus			1	7		5	1				
Cricotopus sylvestris	55						7	2			
Synorthocladius semivirens	24	10	16	1							
Rheocricotopus effusus	92	77	14	12	4	1	2		2		1
Diplocladius cultriger	14	5	4		3	1		1			
Euorthocladius thienemanni	123	34	119	45	15	20	49	20			
Eukiefferiella claripennis	1		4			1					
Eukiefferiella graeci				1							
Eukiefferiella sp.	221	177	36	65	9	9	3	1	4		3
Total	551	337	202	141	40	40	63	31	8	1	4
CATEGORY 6.											
Aedes detritus							1				
Dicranota sp.	9	19	5	15	5					1	
Other Tipulidae	3				4		2	3		1	
Ceratopogonidae (unidentified)				1			1				
Simulium ornatum	1389	703	638	649	279	117	66	53	11	14	12
Hemerodroma sp.	1										
Tubifera sp.		2	1	4	2	2					
Limnophora sp.	14	41	48	68	55	24	51	6	6	5	2
Pericoma sp.		2									
Total	1416	767	692	737	345	143	121	62	17	21	14
Grand total	4037	5300	1507	2296	1260	706	521	218	691	740	949

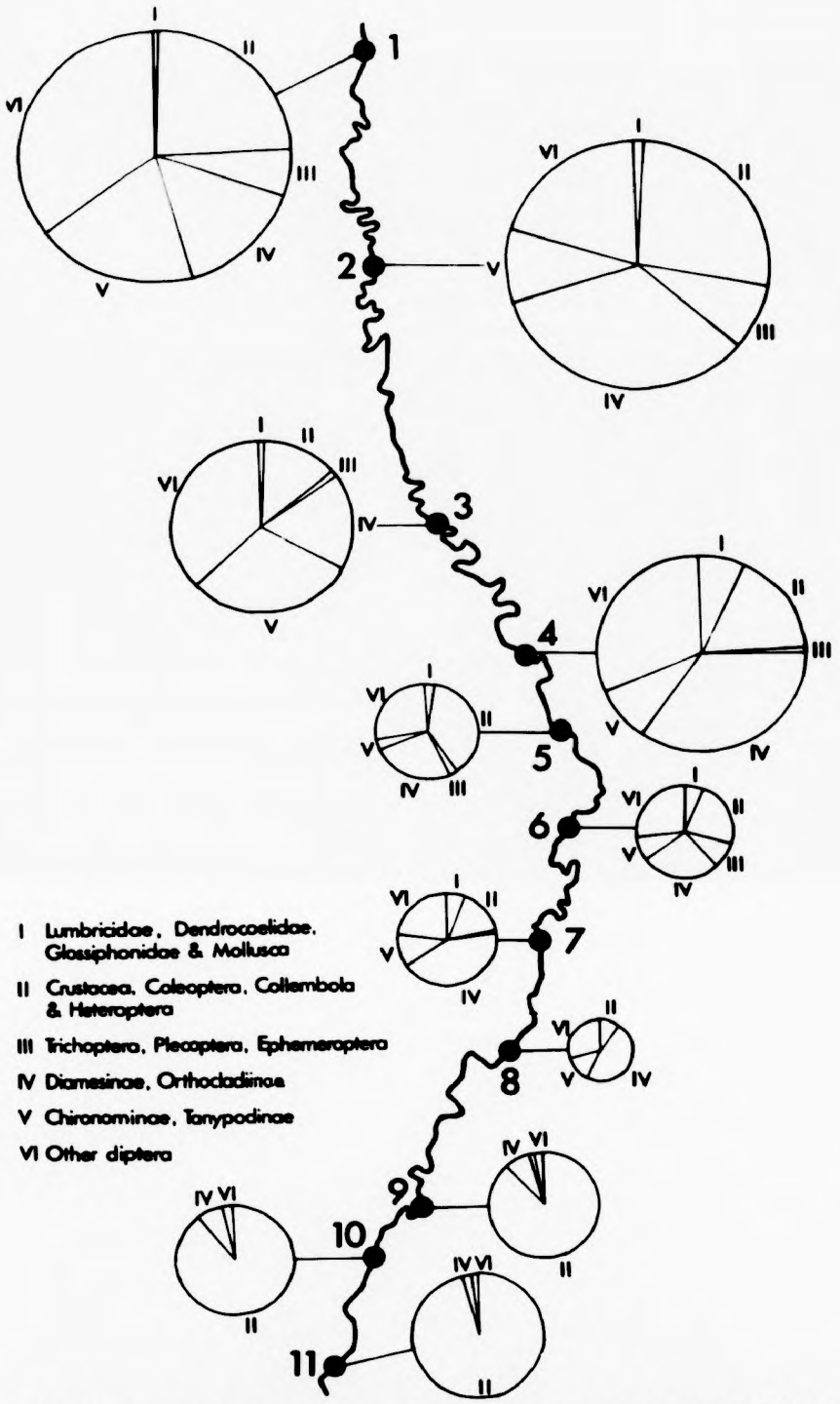


FIG. 9. QUALITATIVE AND QUANTITATIVE COMPOSITION OF THE INVERTEBRATE FAUNA OF CROW BROOK (EXCLUDING TUBIFICIDAE, NAIDAE & ENCHYTRAEIDAE) DIAMETER OF EACH CIRCLE REPRESENTS THE QUANTITY OF BENTHIC ORGANISMS FOUND AT THAT STATION, AND THE SECTIONS REPRESENT THE PERCENT DISTRIBUTION OF DIFFERENT INVERTEBRATE GROUPS.

alternative use of the terrestial environment and their relative impermeability. Their contribution to category II was less than 3%. The third category was composed of the remainder of the non-dipterous insects, the Plecoptera, Ephemeroptera and Trichoptera.

The Chironomidae were divided into two components. As members of the Chironominae and Tanypodinae are notable for their tolerance of salinity (Thienemann 1954) they were allocated to category IV whilst the Diamesinae and Orthocladiinae comprise category V. The final category,VI, contained the remaining Diptera.

Changes in number of species. A dramatic decrease in species abundance was apparent along the stream as salinity conditions became more extreme (fig.10). Stations 1 and 2 of the stream had the richest fauna consisting of 44-45 different species, whereas stations 3-6 had a slightly impoverished fauna of 33 to 37 species. The variety of fauna at station 7 was similar with 31 species, but there was an abrupt decline in diversity by stations 8 and 9 which had only 16 to 20 species. Stations 10 and 11 had the sparsest fauna comprised of only 14 species.

DISCUSSION

1. The freshwater stretches of the stream.

Monitoring of the physical and chemical parameters of the stream indicated that salinity was the only factor likely to affect the fauna. Temperature, water hardness, hydrogen ion concentration and oxygen saturation were all favourable and the fauna of the freshwater stretches was rich both in terms of its diversity and its composition. Use of the Trent Biological System of Stream Classification (Woodiwiss 1964) gave an index of 9, signifying a healthy stream fauna.

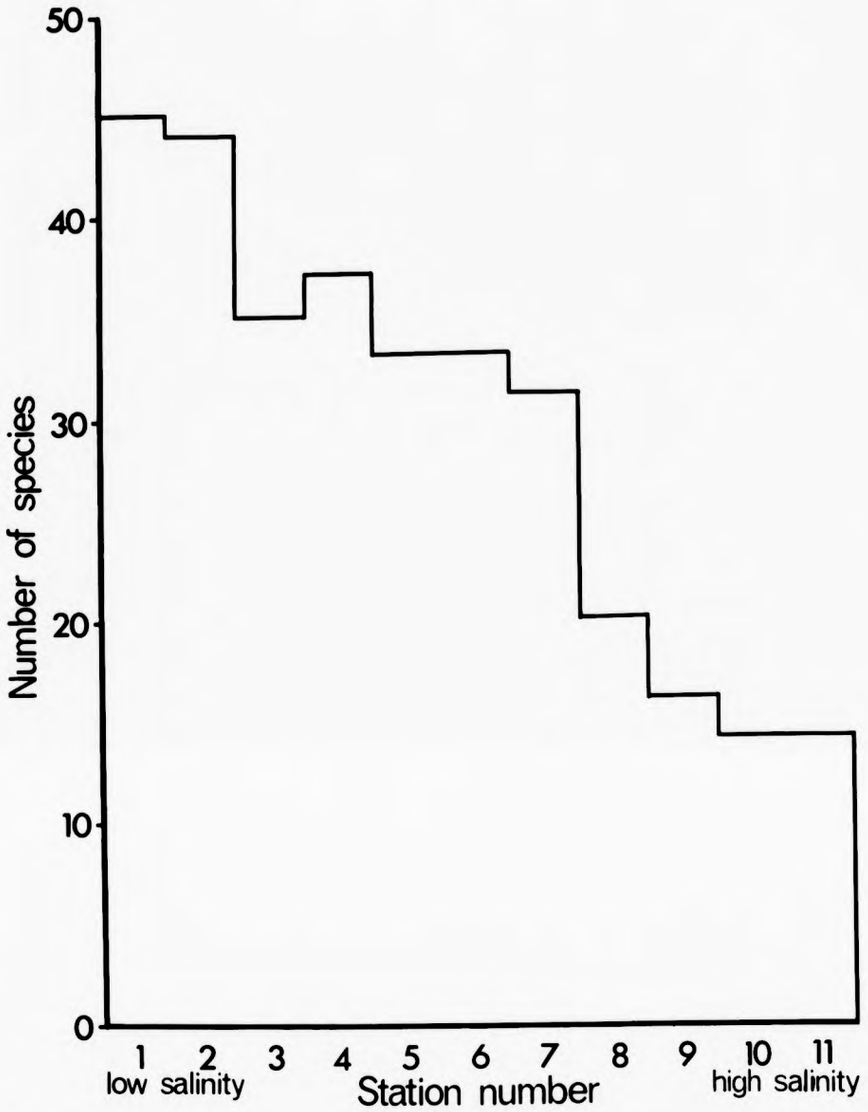


Fig. 10. Number of species at stations along Crow Brook

2. Effect of brine on the fauna.

The changes in the ecology of the stream which were associated with increasing salinity fell into three categories. Firstly there were the changes in the abundance of each species with respect to salinity, secondly there was a decline in the total number of species, and finally, there were changes in the species - numbers relationship.

(a) Distribution and abundance of each species.

Major changes in the synecology of the stream as salinity increases must initially be analysed in terms of the tolerances of individual species, as inferred by their frequency distributions with respect to salinity (fig.8). These tolerances are now discussed for each group in taxonomic sequence.

TURBELLARIA. Polycelis tenuis was the only species recorded and just two individuals were found, at station 2 of the stream.

OLIGOCHAETA. Six species representing five families were present in the stream. Nais elinguis and Lumbricillus reynoldsoni Backlund were abundant along the whole stream whereas mature Tubifex tubifex and Limnodrilus hoffmeisteri, although abundant upstream, declined in numbers at higher salinities and were absent at station 11. Eiseniella tetraedra was found in small numbers as far downstream as station 7 and Lumbriculus variegatus was found in slightly greater numbers as far as station 8. According to Remane & Schlieper (1958) Lumbricidae are very intolerant of salt and E.tetraedra is the only species found in brackish waters. They classify Tubificidae as euryhaline 3^{o}, i.e, reaching 8,000 $mgl^{-1}S$ in the Baltic, and Nais elinguis as being virtually holeuryhaline. L.reynoldsoni is a littoral Enchytraeid with a salinity preference for concentrations somewhat less than half sea water (Tynen,1969). The relative tolerances of the oligochaetes found in Crow Brook are thus in accord with previous records.

HIRUDINEA. Glossiphonia complanata and Erpobdella octoculata were consistently recorded only from stations 1 and 2, though solitary specimens were taken up to station 5 in the case of G.complanata and station 6 in the case of E.octoculata. Only one specimen of Helobdella stagnalis was taken at station 2. The

frequency of leeches therefore apparently declines where mean salinities exceed 1,000 mgl^{-1}Cl in a poikilohaline environment, and are absent where mean salinities are greater than 2000-3000 mgl^{-1}Cl. Pyefinch (1937) found the same three species in the sea-spray pools of Bardsey Island, but did not record them above 400 mgl^{-1}Cl (736 mgl^{-1}S) and Nicol (1938) found that the limit for G.complanata was between 600 and 1000 mgl^{-1}S in the brackish-water lochs of Orkney.

Rawson and Moore (1944) investigated the faunas of inland saline lakes in Saskatchewan and they also found that leeches were vulnerable to raised salinities. Numbers of G.complanata, H.stagnalis, and a Canadian species Erpobdella punctata (Leidy) were significantly lower in those lakes where salinities reached 2,250 mgl^{-1}S or more.

Leeches have been recorded from the Baltic at relatively high salinities, and the range of G.complanata is considered to extend to over 3^{o}/oo S (Remane and Schlieper 1958). Gresens (1928) found that with acclimation the species could tolerate salinities as great as 5.3 ^{o}/oo S. Leeches therefore appear to be more tolerant of salinity in stable environments, such as the Saskatchewan lakes and the Baltic, than in environments where salinity fluctuates, and variability is probably a critical factor governing their range of tolerance in Crow Brook.

MOLLUSCA. Only one specimen of Lymnaea pereger was taken in Crow Brook (station 5) and one of Sphaerium sp (station 2). Ancylastrum fluviatile was recorded once at station 1 and once at station 6. Potamopyrgus jenkinsi was the only mollusc recorded frequently in the stream and was found at stations 4-7. The peculiar history of the snail is informative in the context of its range in Crow Brook. The species, marine in origin is thought to have been introduced to the N.W. European area in recent times and the first record was from the Thames at Plumstead in 1883. Subsequent specimens were initially only taken from brackish waters, but later records showed an extension of the range of the mollusc

into freshwater. Bondesen and Kaiser (1949) considered that it was a species in evolution, this being manifested in the high variability of its ecology, shell structure and reproduction. Although P.jenkinsi is a brackish water form in inland regions of Europe, the tetraploid British form is widely distributed in freshwaters. The mechanism which permitted the species to invade freshwater appears to have become somewhat inflexible and the mollusc no longer seems capable of tolerating high salinities. This is borne out by a number of records, and the range of the species in Crow Brook lends further support to the observation. Bondesen and Kaiser (op.cit) reported that in Denmark the species cannot tolerate salinities greater than $17^{o}/_{oo}S$ and Hubendick (1950) put the preferred range of the British race of the mollusc as between 200 and 1,600 $mgl^{-1}S$, though in the laboratory freshwater specimens could be transferred to $20^{o}/_{oo}S$ without acclimation. The preferred range in Crow Brook is comparable, though the reservation must be made that this could result from a parallel decline in macrophytes on which the snail may graze.

CRUSTACEA. Only two Ostracods were taken, one from station 1 and one from station 4. The distributions of three other species of Crustacea, namely Gammarus pulex, G.duebeni and Asellus aquaticus showed a very distinct interrelationship whereby each species occupied a characteristic part of the salinity spectrum. The optimum salinity, as indicated by maximum abundance, was distinct for each species. Whereas G.pulex was the dominant crustacean in the freshwater zone of the stream, A.aquaticus was dominant at moderate salinities and G.duebeni was the only crustacean taken from stations 9-11.

The absence of G.duebeni from freshwater on the mainland of Britain has been the subject of much debate and Hynes (1956) suggested that although G.duebeni is facultatively a freshwater animal it is only able to become one in the absence of competitors. He suggested that as the distributions of G.duebeni and G.pulex in Britain overlap only in the Isle of Man, competition from G.pulex is the primary limiting factor. He also thought

that there must be some other special requirement of G.duebeni in freshwater, possibly concerned with osmo-regulation. Hynes (1955b) calculated that G.pulex has twice the reproductive capacity of G.duebeni and emphasised this feature as being of critical importance. Sutcliffe (1967c) challenged these calculations and arrived at a reproductive potential of 117-144 offspring per year for G.pulex and approximately 100 offspring per year for G.duebeni. He stresses that mortality figures must also be considered in the interpretation of these results. He further maintains that it is inconsistent that competition from G.pulex restricts the range of G.duebeni,having found a population of the latter in the sea spray areas of the Kintyre peninsula which is separated from mainland populations of G.pulex by an intermediate zone devoid of gammarids. In the laboratory, Sutcliffe (op cit) demonstrated that the brackish water Gammarus duebeni from England have a sodium transport system which is fully saturated with sodium ions when the external medium contains more than 230 mg l^{-1} of sodium and that this presents optimal conditions for its functioning. He found that the system was operating at its maximum rate when half saturated in external media of 46-23 mgl^{-1} sodium and that in even more dilute media the concentration of the blood fell.

In Crow Brook G.duebeni rarely occurred upstream from station 9, (mean salinity 3,340 mg l^{-1}Cl) even though station 8 provided a slightly higher mean salinity and station 7 a mean salinity only 200 mg l^{-1}Cl lower. Salinity minima for the stations, however,

are more informative, those of stations 9-11 being greater than 330 mg l^{-1}Cl and those of the other saline stations being less than 300 mg l^{-1}Cl. It would be expected that the source of sodium ions in Crow Brook is almost entirely dissociated sodium chloride and that the sodium levels of 230 mg l^{-1} required by brackish water G.duebeni would be attained wherever chlorides were greater than 355 mgl^{-1}. Stations 9-11 are the only ones that come close to providing these conditions all year round. This is consistent with the findings of Holland (1976b) who found that the distribution of G.duebeni in the Mersey and Weaver catchment area indicated a minimum salinity requirement of around 500 mg l^{-1}Cl.

It seems unnecessary then to look to competition from G.pulex for an explanation of the exclusion of G.duebeni from the less saline stretches of Crow Brook, and in fact, their distributions do not overlap at any time of the year (fig.8b). Numbers of G.pulex had declined significantly by station 7 and were negligible at station 8. Furthermore this tail of the distribution was comprised entirely of immature individuals whose presence is probably due to drift.

The physiological limitations for G.duebeni in freshwater are still unclear. Although Sutcliffe (1967c) demonstrated a physiological optimum for brackish water G.duebeni in the laboratory, he also examined freshwater populations from the Kintyre peninsula and from Ireland which had regulatory systems that could operate effectively in much more dilute media. The species is

therefore undoubtedly capable of adjusting to freshwater in the absence of G.pulex. Sutcliffe (1970) subsequently demonstrated that individuals from streams on the Kintyre peninsula (9-18 mgl^{-1} sodium) could survive and breed when introduced to a pond containing Windermere water which has less than 5 mgl^{-1} of sodium. The same exercise was not successful when specimens of brackish water origin were used. Sutcliffe (1971) suggests that physiological differences between populations are probably phenotypic in origin but it seems more likely in view of the Crow Brook results that adaptability arises from polygenic control rather than from different expressions of a single genotype. If the same genotype could direct the development of individuals with different regulatory abilities in accordance with the environment, then it would seem unlikely that the less variable low salinity zone of Crow Brook would remain unoccupied by Gammarids. On the other hand, if flexibility in the species arose from linkage of a gene complex, a population would remain fitted to its average environment, whilst regularly producing individuals with more extreme tolerances as a result of cross-overs within the polygene. Whereas a permanent alteration in the environment would allow selection to act for these individuals and to generate a population with the appropriate tolerances, such a system operating in the poikilohaline environment of Crow Brook would be adjusted to the average of the conditions occurring within a generation span. Although individuals capable of inhabiting the low salinity zone would arise as a result of cross-overs, the accompanying changes in other genes would leave the individual less suited to its overall environment. The main population would compete success-

fully with the new genotypes unless the disadvantages of the auxillary genetic changes were outweighed by the advantage of the new regulatory abilities.

It is conceivable that if individuals of the Kintyre population of G.duebeni were already in possession of a gene complex adjusted towards freshwater, they could survive and produce a new population in the totally new conditions of Windermere water. On the other hand, brackish water individuals would need a more radical reorganisation of the gene complex and this would expose auxilliary weaknesses. The populations of G.duebeni which inhabit hypersaline shore pools (Forsman 1956) could similarly originate from populations whose gene complex was adjusted to estuarine or sea water.

A similar argument holds for the restriction of G.pulex to freshwater and low salinities in Crow Brook and other inland saline streams. Hirsch (1918) found that the normal range of G.pulex in the R.Wipper was in salinities below 1.7°/ooS though he found it locally in salinities of 2.8°/ooS at stream confluences. Albrecht (1954), however, failed to find the species outside the freshwater reaches of the river. The species has nevertheless been recorded at higher salinities and Schmidt (1913) recorded it at 5.8°/ooS in Oldesloe, and astonishingly, Thienemann (1913) recorded it at 25°/ooS at salt processing sites in Westphalia. Although the latter record is obviously exceptional, the former indicates that in a stable rather than a poikilohaline environment, selection could act on a gene complex fitting it to the average environment. Certainly the hypothesis of exclusive ranges of G.pulex and G.duebeni arising from competition is untenable in the Crow Brook situation. The adult range of G.pulex does not encroach significantly on that of G.duebeni and the extension of the range of G.pulex into moderate salinities involved only immature individuals. Numbers of both G.pulex and Asellus aquaticus had declined significantly by station 7 and were negligible by station 8, (fig. 8b) indicating that competition for feeding and breeding sites is unlikely.

However, it does appear that A.aquaticus may be a facultative halophye organism in that it becomes more abundant in the zone where numbers of G.pulex have declined. Indeed an Asellus zone is characteristic of organically polluted rivers where the species takes advantage of its greater tolerance to exploit the vacant niches of more sensitive groups (Hynes 1960). A degree of euryhalinity is confirmed by previous records of A.aquaticus in Oldesloe and Westphalia where it has been found in salinities of up to 5.8°/ooS (Schmitz 1959).

INSECTA.

Collembola were found at all stations except number 10, though only once at stations 9 and 11. The sparsity of records downstream cannot be regarded as significant, and in view of the hydrophobic cuticle of Collembola, they are considered here as being independent of the external medium.

Plecoptera were rare in the stream. Two individuals of Nemoura cinerea were taken from station 2, one from station 4, and rather surprisingly, as Plecoptera have been classified as markedly freshwater stenohaline (Remane & Schlieper, 1958), at station 7 in a salinity of 4,400 $mgl^{-1}Cl$. Records such as this are undoubtedly attributable to invertebrate drift which Elliot and Minshall (1968) have shown to be considerable in streams of this type.

Ephemeroptera were represented by Baëtis rhodani alone. Large numbers were found at the two freshwater stations, but an abrupt decline in abundance was seen at stations 3, 4 and 5 (fig. 8c). Numbers were negligible at station 6 and nymphs were absent from downstream stations. Ephemeroptera are generally regarded as being sensitive to salinity (Rawson and Moore, 1944; Remane & Schlieper, 1958) and neither Hirsch (1918) nor Albrecht (1954) found any Ephemeroptera in salinities greater than 2.8°/ooS in the Wipper. Johannsen (1918) regarded B.rhodani as an exception, having found it at 1,500 $mgl^{-1}S$ and my results confirm his opinion.

Of the nymphs recorded at stations 3-7, ten were from salinities above 1,500 $mgl^{-1}Cl$ (2,520 $mgl^{-1}S$) and six from salinities above 2,000 $l^{-1}Cl$ (3,360$mgl^{-1}S$) indicating that B.rhodani displays a degree of euryhalinity in Crow Brook.

Trichoptera. The predominant caddis in the stream was Hydropsyche angustipennis, larvae of which were abundant in association with H.pellucidula at stations 1 and 2. H.angustipennis appeared somewhat more tolerant of salinity than H.pellucidula which was only recorded at stations 1 and 2, while H.angustipennis was recorded in low numbers at stations 3-5 and, extraordinarily at station 11 (fig.8c). H.pellucidula is also less tolerant of pollution and low concentrations of dissolved oxygen than H.angustipennis (Badcock, pers.comm., Philipson and Moorhouse, 1974), and this overall sensitivity may relate to levels of activity. Investigations conducted by Claus (1937) indicated that in Corixidae at least, stenohaline species have an increased oxygen consumption which he attributed to the amount of work needed for the maintenance of internal osmotic concentration. A high oxygen demand could necessitate an increased water flow over the gills of the larva and one would expect this to increase vulnerability to osmotic damage.

Another common caddis in the stream was Hydroptila tineoides which appeared to be localised and abundant, occurring only at stations 2, 5 and 6. As the first four instars of this species are naked, (Hickin 1967) plant cover may be an important factor in their distribution. Albrecht (1954) found the genus in the R.Werra where it was euryhaline tolerating salinities up to around $6^{o}/_{oo}S$. Occasional specimens of Potamophylax sp. were recorded at stations 1 and 2, and 5-7.

Coleoptera. Although only three specimens of adult Cercyon sp. were taken, beetle larvae were recorded at intervals all along the stream and were thought to be the same genus. Elmis aenea was found at stations 1 and 2 but numbers were low and these limited records were not thought to indicate stenohalinity.

Chironomidae. This was the most diversely represented group in the stream and larvae and pupae of 26 species were recorded.

Five species of the Tanypodinae were present, though only two of them in any significant numbers. Pentaneura sp was recorded principally at stations 1-4 and this was the apparent limit of its range of tolerance, though it is unclear whether this is a physiological or ecological limit. Single individuals were taken at stations 5 and 6 and two individuals at station 7. Macropelopia sp. was taken at every station except 11 and numbers were greater at stations 4-9 than in the rest of its range,(fig.8d) indicating a halophye tendency. The sparse records of the very closely related Apsectrotanypus trifascipennis seem to show the same trend. The downstream stretches of the stream are possibly more advantageous for these two species due to the reduction in numbers of Pentaneura sp.and other Tanypodinae, all of which are potential predators. Psectrotanypus varia and Procladius sagittalis were also recorded from the stream at station 2.

A typical rheophilic fauna of Orthocladiinae was found and was composed of 10 species. The distributions of most of these were concentrated in the low salinity stretches of the stream,(fig. 8e-f). Of the 51 individuals of Synorthocladius semivirens that were found, only one was taken downstream from station 3. Brillia modesta, Eukiefferiella claripennis, Eukiefferiella sp. and Rheocricotopus effusus were not very euryhaline, with the majority of the records coming from stations 1-4. Diplocladius cultriger was recorded less frequently, but shows a similar pattern and records of Cricotopus sylvestris and Eukiefferiella bicolor were even more infrequent. The apparent stenohalinity of the group is not surprising in either ecological or physiological terms. With the exception of the genus Cricotopus and some specialised halophilic forms, very few Orthocladiinae are reported from saline habitats. (eg.Thienemann 1915, 1954). Furthermore, as the group is principally

phytophagous, feeding on plants and plant remains, or trapping algae and detritus in salivary networks, it is probably restricted by a reduced food supply in the saline stretches. Two species do show a less restricted range, Metriocnemus atratulus and Euorthocladius thienemanni. Although records are sparse M.atratulus appears to occupy a somewhat halophye position, and as it is normally a species of cold, turbulent mountain streams one can envisage that outside its preferred habitat it would be sensitive to competition from other rheophilic Orthocladiinae and it may here take advantage of their decline. Furthermore, there is evidence from other inland saline waters that the genus is somewhat euryhaline and Thienemann (1954) reports a record from 5.6°/ooS in Oldesloe and one from 7.3°/ooS in Westphalia. A more extensive distribution was seen for E.thienemanni in Crow Brook which had a range of tolerance clearly extending to station 8. E.thienemanni may be physiologically more tolerant than the other species or availability of food may extend further downstream than for other species.

Three species of Diamesinae were found; Prodiamesa olivacea, Potthastia longimanus and Potthastia gaedi. The first had a distribution similar to that of E.thienemanni covering a wide range of salinity. Although only a few specimens were taken, these came from all stations between 1 and 9 indicating a range consistent with that found in Oldesloe where the larvae were found in salinities greater than 5.6°/ooS (Thienemann 1954). All fourteen specimens of Potthastia longimanus were taken in low salinities at stations 1-4, and this is consistent with the ranges of the majority of the Orthocladiinae. Only a single specimen of Diamesa gaedi was recorded.

Chironominae in the stream came from three genera, Polypedilum, Microtendipes and Chironomus. Three species of Polypedilum were taken, but all were infrequent. The distribution of the three species combined, indicates a successful range extending from station 1 (maximum abundance) to somewhere in the region of stations 4-6. Thienemann (1954) also considered the genus to be

somewhat stenohaline. Microtendipes sp.was extremely abundant in the range extending from station 1 to station 7. In terms of abundance, the distribution of the species was limnetic in bias in summer and yet halophye in winter (maximum abundance at station 4). This effect could be produced by differences in relative intensity of predation between summer and winter. It can be envisaged that a predator which could effectively cull a small overwintering population of Microtendipes sp, may make little impact on its abundance in summer if fecundity of the prey outstrips that of the predator. If the predator were stenohaline, the distribution of the prey would become halophye at those times of the year when relative intensity of predation was highest. Microtendipes sp. is an established component of the diet of Tanypodinae (Macan 1962) and Pentaneura sp. may fulfill the role of predator in this case, though there is little doubt that by virtue of its great abundance, Microtendipes sp. would be a popular component of the diet of any stenohaline carnivore in the stream.

Chironomus anthracinus, C.plumosus, C.einfeldia and C.riparius were all recorded from Crow Brook, but only the last in any substantial numbers. C.riparius is very clearly a halophye organism, having a maximum abundance between stations 5 and 8, and in the winter this tendency is so marked that it appears as an exclusively brackish water animal. The osmotic tolerance of C.riparius is well known and Sutcliffe (1959) demonstrated that the larvae could survive in an external medium of up to 14^{o}/ooS in laboratory conditions. This physiological tolerance is borne out by ecological records of the species and Sutcliffe himself (op.cit) found it at salinities of 1^{o}/oo - 8-10^{o}/ooS at Seaton Sluice. Thienemann(1954) reports that the species was found at an extraordinary salinity of 59^{o}/oo in Westphalia. The species has also been recorded from the Baltic where it was restricted to waters of less than 8^{o}/ooS. (Remane & Schlieper 1958). C.riparius is a detritus feeder and the summer distribution may

correlate with the distribution of Enteromorpha intestinalis which traps an abundance of organic matter and which would also provide shelter from predation. As with Microtendipes sp. the seasonal change in distribution may be attributable to predation.

Tipulidae. Larvae of Dicranota sp. were collected at stations 1-5 (fig.8a), and this seems to be the limit of their range, although one individual was taken at station 10. Small numbers of other Tipulidae were recorded infrequently along the whole stream.

Culicidae. One larval specimen of Aedes detritus, a genuine brackish water mosquito, was taken at station 1 in the stream and this is probably a stray, as the imagines are extremely common over the brinefield where they breed in the brine reservoirs.

Ceratopogonidae. Only two larvae of this family were recorded, although sampling techniques may have led to the loss of the small species of this group.

Simulidae were abundantly represented by the larvae of Simulium ornatum whose distribution was wide with a clear bias towards freshwater. The bulk of the records were from stations 1-6.

Syrphidae. Two species were present; specimens of Pericoma sp were taken only twice and Tubifera sp was a little more frequent having been taken at stations 2-5.

Muscidae. Large numbers of larvae of Limnophora sp were taken from the stream. The species occupies a clear halophye position with a maximum at station 4(fig.8a). This is again attributed to predation from limnetic carnivores as the larvae are found in an exposed niche, actively crawling over Fontinalis antipyretica in substantial numbers. The decline of the species in the saline stretches may not be due to salinity per se, but to the decline in

F.antipyretica. Certainly the genus is a very tolerant one and the species L.litorea was recorded at 105°/ooS in Westphalia by Thienemann (1913).

ARACHNIDA. Eleven specimens of Hydracarina were taken, though due to their small size many may have been lost in sampling. None were found beyond station 8 and this may indicate some lack of tolerance of elevated salinities.

Classification of species in relation to brine tolerance. If the range and salinity optimum is considered for each species, it is possible to assign species to various categories. As the faunal survey had revealed no major discrepancies between the distribution of species in Crow Brook and their known tolerance for salinity, this allocation could be done with a high degree of confidence, once allowance for any bias of the range had been made. It was apparent that in certain circumstances the apparent distribution of a species would exceed its range of tolerance. Firstly it must be assumed that moribund individuals would have been included in the distribution figures at stations downstream from their actual range of tolerance in situations where salinity had increased very recently. Secondly it is apparent that invertebrate drift would artifically enrich a saline zone, (Elliott & Minshall 1968) and that this effect would be exaggerated where individuals which were already at the limits of their tolerance were incapacitated and more liable to drift. A correction was made for these influences by applying less weight to the extreme 10% of any individual distribution. Thus the single specimen of H.angustipennis taken at station 11 (fig.8c) was not considered. Numbers were low for several species, but where the distributions seem representative of the range of tolerance, they have been included in the faunal classification. Records of closely related groups have been considered as clusters in three cases, those of the Hirudinea, of Polypedilum spp. and of Anatopynia spp.

The categories to which species were allocated are first defined

in table 5 where the terminology used is that of Remane and Schlieper (1958). Salinity limits for each category are given in table 7 and are somewhat lower than those used by Remane and Schlieper in view of the variability of salinity in Crow Brook.

The species lists given in table 5 are presented in the appropriate categories, with two additional categories, one for those species whose distribution is deemed independent of the aquatic environment and one for those species for which records were inadequate to indicate a range of tolerance. The validity of this biological classification is in part confirmed by its accord with previous records from inland saline waters, though ultimately it can only be justified by its predictive accuracy. These categories will subsequently be used for the construction of a halobic index whereby severity of a saline environment can be rated according to the richness and composition of its fauna.

(b) Changes in number of species.

It is apparent then, that as salinity increases, progressively more species decline in abundance and are eventually eliminated, and this progression is reflected in a declining histogram of number of species (fig.10). The histogram takes the form of a saltus with discontinuities between stations 2 and 3 and between stations 7 and 8 indicating that the probability of stenohaline limnobionts being found beyond station 2 drops dramatically. The decline between stations 7 and 8 similarly indicates a point at which the probability of occurrence of euryhaline limnobionts 2^{o} and of halophye organisms decreases. Thus, while the discontinuities cannot be used to describe specific faunal elements, they can be used to subdivide the stream into three distinct environments.

The singular richness of the fauna at stations 1 and 2 confirms that these stations belong to a distinct zone, which is obviously a freshwater zone, having an upper salinity limit somewhere between mean salinities of 200 and 1500 $mgl^{-1}Cl$ (340-2,520 $mgl^{-1}S$). As the decline in species diversity between stations 3 and 7 is

Table 7. Degree of tolerance of various categories of invertebrates in Crow Brook.

Category	Definition	Stations at which recorded.	Range of mean salinity (mgl^{-1})
Stenohaline limnobionts and euryhaline limnobionts of the first degree.	-strictly freshwater species and species extending from freshwater to waters of low salinity.	1 - 4	Freshwater to 1600 Cl (2690 S).
Euryhaline limnobionts of the 2nd degree.	- species extending from freshwater to waters of moderate salinity.	1 - 6	Freshwater to 2200 Cl (3700 S).
Euryhaline limnobionts of the 3rd degree.	- species extending from freshwater to waters of high salinity.	1 - 9	Freshwater to 3300 Cl (5544 S).
Halophye organisms of 1st degree.	- freshwater species which attain maximum abundance in moderate salinities.	Maximum between 4 - 7	1600 - 3100 Cl (2690 - 5210 S).
Genuine brackish water organisms.	- those species apparently confined to briny water, many of these are mixohaline.	8 - 11	3100 - 4200^{+}Cl (5200 - 7060^{+}S).
Holeuryhaline.	- those species whose distribution ranges from freshwater to waters of extreme salinity.	1 - 11	freshwater to extreme salinity.

slight, these can be grouped on biological grounds into a region, contiguous with the freshwater zone, and extending to mean salinities of 3,140 mgl^{-1}Cl (5,280 mgl^{-1}S). Stations 8-11 have an impoverished fauna and must also be amalgamated into one region which extends from mean salinities of around 3,560 mgl^{-1}S) to mean salinities in excess of 4,190 mgl^{-1}Cl (7,040 mgl^{-1}S).

In Venice, the Symposium for the Classification of Brackish Waters (Anon, 1958) considered biological parameters and in particular faunal diversity in brackish waters and arrived at an empirically based system for the subdivision of marine-brackish waters. The more dilute zones were the 'limnetic' which ranged from freshwater to 500 mgl^{-1}S, the 'oligohaline', which ranged from 500-5,000 mgl^{-1}S, and the 'mesohaline' which ranged from 5,000 to 18,000 mgl^{-1}S. The two discontinuities in faunal diversity in Crow Brook correspond well to the limnetic/oligohaline and oligohaline/mesohaline boundaries delineated at the Venice Symposium, and these are obviously natural boundaries for a freshwater fauna. Mean salinity is therefore a useful parameter with which to define a poikilohaline environment of this type, though the reservation must be made that fluxes of salinity are probably relatively brief in Crow Brook, and that invertebrate drift is acting towards exaggerating the salinities at which the natural boundaries occur. Duration of salinity fluctuations must generally be important and it cannot be meaningful in biological terms to express poikilohalinity in terms of a range or standard deviation of salinity about a mean, and for this reason a biological method of assessing the severity of a saline environment would be a useful tool.

Decline in number of species is one obvious indicator of increasing salinity, and if the changes in individual faunal elements are also considered, it is possible to construct a matrix which can be used to rate the severity of a saline environment. Table 8 is a two dimensional matrix, similar to that used by Woodiwiss (1964) for rating polluted waters. In the matrix presented here, a low index is used to signify a severe saline environment and a high one to signify a healthy environment. An increase in

Table 8. Biological system for the classification of saline waters.

Freshwater	Classification according to table 5.	Total number of species present.					
		0-5	6-10	11-19	20-29	30-39	40+
		Halobic index					
Changes in representation of groups as salinity increases.	Stenohaline limnobionts > 20% of species found.	-	-	-	18	19	20
	Stenohaline limnobionts > 5% of species found.	-	-	16	17	18	19
	Stenohaline limnobionts present, but < 5% of species found.	-	12	13	14	15	16
	Stenohaline limnobionts absent, but euryhaline 2^{o} species > 10% of species found.	-	9	10	11	12	13
	Euryhaline 2^{o} species < 10% of species found. Halophye < 50% of species found.	-	6	7	8	9	10
	Euryhaline 2^{o} species absent. Halophye and euryhaline 3^{o} species > 50% of species found.	4	5	6	7	8	9
	Species other than halophye, brackish water and holeuryhaline species still present.	3	4	5	6	7	8
	Only halophye, brackish water, and holeuryhaline species present.	2	3	4	5	6	7
	Holeuryhaline species only.	1	2	-	-	-	-
Hypersaline							

severity is manifested by both a decline in number of species (taken from right to left across the page) and in a change in the composition of the fauna whereby salinity tolerant species become a progressively more important component of the fauna. To derive the halobic index for a saline habitat the fauna must be analysed in terms of these two parameters. The appropriate description of the fauna must be selected from the column of successive changes in faunal composition, and the halobic index must then be chosen according to the number of species present.

The sequence of changes listed from top to bottom of table 8 are empirically based, having been derived from an analysis of the changes in the fauna of Crow Brook with increasing salinity. The matrix, although derived from an analysis of the Crow Brook fauna, subsequently proved appropriate for the other saline habitats that were investigated, though provision had to be made for very extreme saline environments by introducing the three most tolerant groups to the table. The sensitivity of the halobic index in discerning salinity differences at these other habitats (eg. see p.220) testified to the validity of the method.

(c) Changes in the relationship between number of species and numbers of individuals.

Although the major change in the community structure along Crow Brook is the decline in the number of species, this is accompanied by significant changes in the total abundance of invertebrates (fig.9). It is typical of many forms of pollution that total invertebrate numbers decline progressively until pollution tolerant species are freed from interspecific competition and consequently begin to proliferate in numbers (Hynes 1960). Food chains may also change and simplify, producing the same effect (Koryak et al 1972). The index of diversity, α (Fisher et al 1943), can be used to quantify this trend in Crow Brook (Table 9). ($S=\alpha \log_e(1+\frac{N}{\alpha})$ where S=number of species, N=number of individuals).

Table 9. Index of diversity of fauna at stations along Crow Brook.

	low salinity								high salinity	
station no.	1	2	3	4	5	6	7	8	9	10	11
α	7.1	7.0	6.0	6.5	6.2	7.9	7.3	5.5	2.8	2.5	2.4

The fall in the index at station 8 indicates the initial proliferation of numbers of salt tolerant species and this imbalance in the species-numbers relationship becomes extreme by stations 9-11 which have an extremely low index of diversity. The same effect occurs in the Baltic where the number of species is very low in moderate salinities and yet Chironomidae and a few other forms give total numbers of up to 7,600 individuals in a square metre (Segerstråle 1949).

Changes in the contributions to the fauna of the major taxa account for the changes in diversity which have been discussed in detail in the previous two sections. The first major change in the composition of the fauna (fig.9) is the loss of the Plecoptera, Ephemeroptera and Trichoptera by station 6, and this is followed by the loss of the large annelids and the molluscs at station 7. By station 9 the contribution of the Orthocladinae and Diamesinae to the fauna is negligible, and in the stretches of higher salinity the rest of the Diptera represent only a small proportion of a fauna totally dominated by the Crustacea. The increasing contribution of the Crustacea to the total fauna of the saline stretches is entirely due to the contribution of Gammarus duebeni (see table 6.) G.duebeni shows facultative holeuryhalinity (Remane and Schlieper 1958) and here it is apparently exploiting a situation where there is no competition from other Crustacea nor indeed from any other invertebrates. G.duebeni is in an advantageous position in Crow Brook whereby it is in a saline environment which is inaccessible for other brackish water organisms. G.duebeni itself probably migrated through the R.Weaver which receives heavy effluents of calcium chloride, and through the badly polluted Wade Brook to reach Crow Brook.

The rapid decline of other taxa along Crow Brook illustrates the general rule that freshwater organisms having become adapted to living in an osmotically extreme environment, scarce in ions, are consequently endowed with inflexible regulating abilities. The Diptera, however, being primarily terrestial, solved the problem of dilution of body fluids in a freshwater environment by virtue of both their less permeable cuticles and their tolerance of

alterations in the internal medium, rather than solely by active uptake if ions. Thorpe (1931) confirms this, "Diptera are not only able to support life in a medium of an osmotic pressure that is rapidly fatal to most other insects, but they have also a greater power of adaption to changes in concentration than have other orders". It is hardly surprising then that the Diptera penetrate further into the saline stretches of Crow Brook than other groups of insects.

SUMMARY

1. The ecology of a stream contaminated by brine was studied in relation to its salinity which increased progressively downstream, from freshwater to a mean salinity of 4,190 $mgl^{-1}Cl$ (7,040 $mgl^{-1}S$). Variability of the salinity was high.

2. Sixty five species of invertebrate were recorded from the stream and the distributions of the more abundant groups are discussed in relation to salinity. The dominant freshwater species are categorised either in relation to their degree of euryhalinity or to any halophye tendency that they displayed.

3. The number of species declined with salinity and indicated three distinct zones within the stream which are taken to correspond to the limnetic, oligohaline and mesohaline zones of the Baltic Sea as designated by the Symposium in the Classification of Brackish-waters (Anon 1958).

4. Total numbers of individuals per unit area declined initially as salinity increased, but then increased again as some halophye species and the brackish water Gammarus duebeni became progressively more successful. The index of diversity fell when mean salinities exceeded 3,140$mgl^{-1}Cl$ (5,280 $mgl^{-1}S$) confirming a proliferation in numbers where only a few salt tolerant species remained.

5. The changes in the composition and diversity of the fauna at stations of increasing salinity were used to construct a tentative halobic index.

CHAPTER IV

THE EFFECTS OF A SALINE EFFLUENT ON THE ECOLOGY OF THE TRENT AND MERSEY CANAL.

INTRODUCTION.

The canal investigated in this study traverses the Cheshire salt-fields and received a brine effluent which raised its salinity to about one-fifth that of sea water. This provided a unique situation in which to investigate the changes in an essentially lacustrine fauna in response to elevated salinities.

Previous investigations of the faunas of mixohaline waters in Britain have generally been confined to coastal locations where tidal fluctuations influence the fauna. Even though the faunas of such environments inevitably include animals of marine origin, usually a number of animals of freshwater origin, particularly insects, are also represented.

Butler and Popham (1958) made an extensive survey of the aquatic insect fauna of the Spurn Peninsula, both before and after the excessively high tides of 1953 and drew conclusions regarding the tolerance of over fifty insect species to elevated salinities. Sutcliffe (1959) studied the salt-marsh fauna at Seaton Sluice and investigated the osmoregulatory responses of a number of insects to raised salinity and Nicol (1933, 1935) investigated the faunas of a number of salt-marshes in Northumberland. Pyefinch (1937) surveyed the faunas of the brackish water pools of a stream outlet on Bardsey Island and established the distribution of a number of freshwater invertebrates. Howes (1939) studied the fauna of a lagoon in a brackish creek in Essex and found a number of freshwater insects amidst a principally marine fauna.

On the Continent, studies of the Baltic Sea (Johannsen 1918, Lindberg 1948, Remmert 1955b, Forsman 1956, Segerstråle 1957) have provided a wealth of information about the ranges of freshwater

invertebrates in relation to salinity and many of the species are common to Britain. In the Netherlands Redeke (1935) catalogued the brackish water fauna of the Zuiderzee before the barrage and Beaufort (1953) has reviewed studies of changes in the fauna of Ijsslemeer as it became progressively more dilute. Remane and Schlieper (1958) review brackish water studies from these and a variety of other sites.

The present survey contributes a new aspect to the study of the ecology of mixohaline waters, and although the canal is, strictly speaking, briny rather than brackish, it would be pedantic to resist comparisons between its fauna and those of Ijsselmeer and the Baltic which are also free from tidal influx and relatively isolated from the open sea.

Genuine brackish water species, and in particular Gammarus spp, have received rather more attention than freshwater species which occur in mixohaline environments. Holland (1976a, b) studied the Crustacea of the Trent and Mersey Canal. Hynes (1955a, 1956) discussed the distribution of Gammarus spp.in Britain generally, and Hynes (1955b) compared their reproductive potentials in an attempt to elucidate their relative distributions. Kinne (1952, 1954, 1964) discussed the factors affecting the distribution of Gammarus zaddachi and G.duebeni with particular reference to Baltic populations, and Sutcliffe (1968) examined differences in sodium regulation in British Gammarus spp. The distribution of G.duebeni in particular is discussed by Sutcliffe (1967c, 1971) and the biology and ecology of the species has been investigated by Kinne (1953, 1959) and Hynes (1954). In the Netherlands the effect of the arrival of the alien G.tigrinus on the indigenous populations of G.zaddachi and G.duebeni has been discussed by Smit (1974) and Pinkster (1975).

The present study contributes some new aspects to the discussion of the relative distributions of the mixohaline Gammarus spp. in Britain.

DESCRIPTION OF THE CANAL AND LOCATION OF SAMPLING STATIONS.

The Trent and Mersey Canal originates at Derwent mouth on the River Trent and passes through Stoke to flow northwards across the Cheshire Salt Fields (fig.1 p2) to join the Mersey river system via the Bridgewater Canal (fig.11). The canal at one time received several effluents of waste brine from the salt processing factories in the Sandbach and Middlewich area, but since 1968 only one of these discharges remains, entering the canal just below Elworth. Total salinities reach nearly 20% seawater between Elworth and Middlewich where the entry of the Middlewich Link Canal effects considerable dilution. Run-off and land drainage contribute progressively to dilution until the junction of the canal with the Bridgewater Canal. The latter also has moderate salinities which arise at Lymm where the brine table reaches the surface.

An ecological survey of the canal system was made over a period which included the last month of 1971 and the whole of 1972, though observations were restricted almost entirely to the Trent and Mersey Canal itself, owing to the limited time available. Sampling points were selected to give coverage of the saline stretches as far as the junction of the Trent and Mersey Canal with the Bridgewater Canal (fig.11). The latter was only sampled on two occasions. Four sampling stations were established upstream of the brine discharge on the Trent and Mersey Canal, and one on the Middlewich Link Canal before it effected the dilution of the saline stretches of the main canal. It was hoped that samples from these points would give some indication of the potential natural fauna of the rest of the canal system. Ten sampling points were used on the saline stretches of the canal.

The canal was on average 6-8m wide and 1.0-1.2m deep at all stations except station 5 which was barely 0.3m deep. All stations had walls constructed of sandstone blocks except stations 1 and 6 where the walls had been cemented. Tree cover was absent from all stations except 1 and 11.

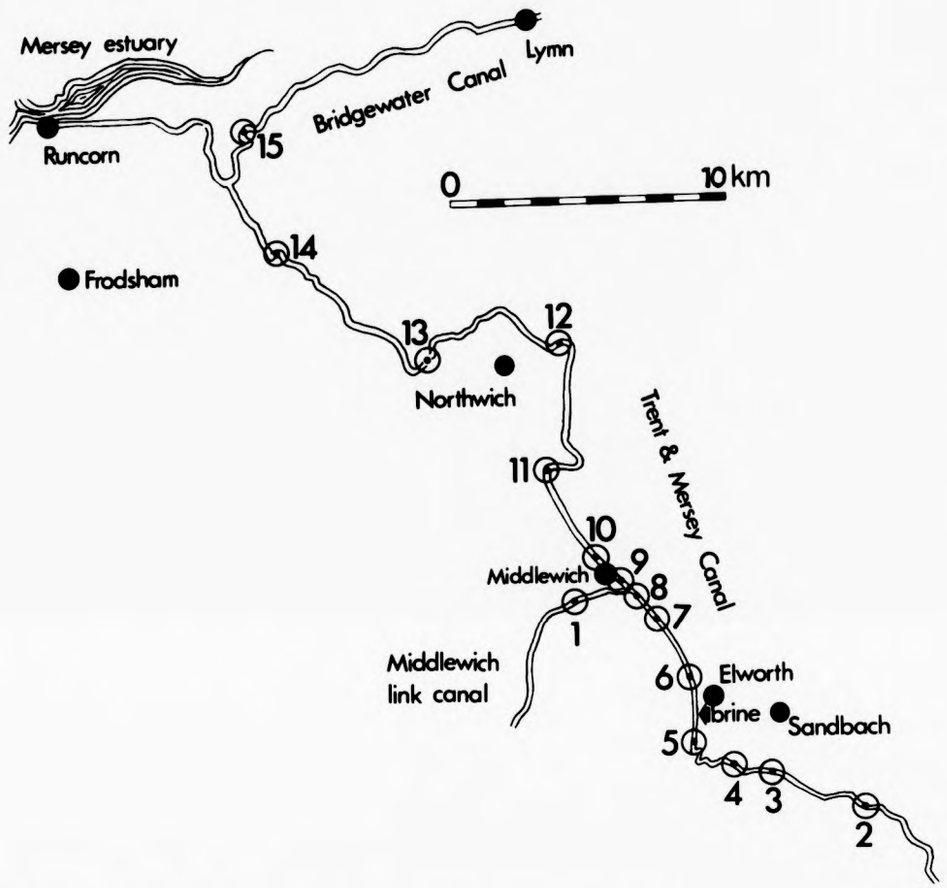


Fig. 11. Map of the Trent and Mersey canal system.

METHODS

Sampling programme.

Biological samples and water samples were taken from the 15 stations on the canals at various times during the survey (table 10). Initially only 7 stations had been used, but as expertise increased, samples were analysed more rapidly, and more sampling points could be added. Road closures prevented the use of station 1 on two occasions, and drainage of the canal for repairs near Cledford Bridge prevented the use of station 7 for 5 months. Large sections of both the Trent and Mersey Canal and the Middlewich Link Canal were drained for repair work in December 1972, and with an increasing research load at other sites this proved a convenient time to terminate the survey.

Water quality.

Water samples from each station were analysed for salinity, pH and conductivity after every visit and for calcium and magnesium on 24.11.71 and 18.7.72. Samples taken from stations 1 and 2 and 5-9 on 24.11.71 were analysed for sodium and potassium, and a more detailed chemical analysis of samples taken at Northwich in August 1972 was supplied by I.C.I. Oxygen saturation was measured at stations 2-6, 8-10 and at station 15 on 18.5.72. Separate samples were taken from surface and bottom waters on 19.1.73 to test for stratification of brine at stations 2 and 5 and stations 9-12.

Physical factors.

The temperature of the surface water was taken regularly at each station, the stretch from station 2 to station 8 being sampled in a downstream direction in the mornings, and that from stations 9-15 and station 1 in the afternoons. Rates of flow were provided by British Waterways Board.

Biological methods.

It was apparent that to obtain a representative sample of the fauna both the floor of the canal, and the walls had to be sampled.

Table 10. Sampling programme for Trent and Mersey Canal survey.

Station No.	Grid Ref. (Square SJ).	Dates Sampled. 24.11.71	17.1.72	7.2.72	9.3.72	6.4.72	18.5.72	15.6.72	18.7.72	17.8.72	29.9.72	30.10.72	14.11.72	8.12.72	Locality.
1	688657	x	x	x	x	x		x	x		x	x	x	x	Stanthorne
2	783583	x	x	x	x	x	x	x	x	x	x	x	x	x	Hassall Green
3	752593				x		x	x	x	x					Wheelock
4	743594				x		x		x	x					Lightly Hill Farm
5	731603	x	x	x	x	x	x	x	x	x	x	x	x	x	Rookery Bridge
6	722638	x	x	x	x	x	x	x		x	x	x	x	x	Tetton Bridge
7	711649	x	x						x	x	x	x	x	x	Cledford Bridge
8	707657	x	x	x	x	x	x	x	x	x	x	x	x	x	King's Lock
9	705663	x	x	x	x	x	x	x	x	x	x	x	x	x	Town Bridge
10	696671			x		x	x	x	x	x	x	x	x	x	Croxton Bridge
11	679697			x	x	x	x	x	x	x	x	x	x	x	Whatcroft Hall
12	685748								x	x		x	x	x	Wincham Mill
13	628752								x	x	x	x	x	x	Barnton
14	584780							x	x	x	x	x	x	x	Dutton
15	572826						x	x							Daresbury

A sampling net was designed with a two-position blade at the net mouth which could be lowered to enable a scrape to be made of the floor of the canal, and then raised to make a superficial scrape of the wall. The area sampled could only be roughly quantified as débris on the canal floor and the irregularity of the walls were a considerable hinderance. Approximately $0.2m^2$ of the canal floor and $0.1m^2$ of the wall were sampled on each occasion. The contents of the net were transferred to a 4 litre container for transportation to the laboratory.

There were no macrophytes at any sampling station and observations on the flora were restricted to rough assessments of type and abundance of algae.

RESULTS

Water quality.

Salinity.

Parameters for each station are detailed in table 11, and mean salinities are plotted with their standard deviations for each station in fig.12. Base levels of chlorides in both the Trent and Mersey Canal and the Middlewich Link Canal lay between 30-100 $mgl^{-1}Cl$. The brine discharge at Elworth raised the chloride levels to an average of 4,640 mgl^{-1} with a range of at least 1,700 $mgl^{-1}Cl$ at station 6. Generally, the salinity of the canal declined with distance downstream, and by station 14 the mean salinity had fallen to 2,590 $mgl^{-1}Cl$. The recorded range of the salinity at any one station is probably underestimated due to the infrequency of sampling. The discharge of brine was intermittent and flow was variable, primarily because of industrial abstraction and compensation, but also because of the lock system of the canal.

No seasonal variations in salinity were apparent (fig.13) and no significant stratification was detected at any station ($\pm$ 2‰). Differences in the fauna along the freshwater stretches raised doubts about whether the stations just upstream of the effluent were contaminated. A t test between station 2 and station 5 revealed no significant differences between their salinities (appendix 2).

Conductivity.

The regression of conductivity (y) against chlorinity (x) is decribed by y=0.0027x + 1.4254, (r=0.96), for stations 6-14 indicating that chlorinity is a reliable indicator of total salinity. The relationship between conductivity and chlorinity was much weaker for the freshwater stations (r=0.33) and the gradient of the regression line much lower as other ions made a relatively greater contribution to total salinity. The relationships are plotted in fig.14.

Hydrogen ion concentration

This is presented in terms of pH in table 12. No pH less than 7.2 was recorded, though levels of over 9 were commonly found throughout the canal system and particularly in summer.

Dissolved oxygen.

The canal was found to be supersaturated at stations 5, 10 and 15 on 18.5.72, and between 70-90% saturated at the remaining 6 stations (table 13).

Other ions.

The results of the analyses of samples taken on 24.11.71 and 18.7.72 are presented in table 14. Total hardness of the water from the Trent and Mersey Canal was between 96-160 mgl^{-1} and that of the Middlewich Link Canal about 70 mgl^{-1}. Data from 18.7.72 indicate that the brine had a slight softening effect, possibly due to the precipitation of calcium carbonate. Levels of sodium and potassium ions reached moderate proportions in the freshwater stretches, and sodium was also higher than would be expected from the dissociation of sodium chloride in the saline stretches. This is almost certainly due to precipitation of chlorides resulting in an increase in the sodium:chloride ratio.

Chemical analyses received from I.C.I are presented in table 15, and information from the North West Water Authority suggests that an intermittent discharge of iron oxide occurs just upstream from the brine outlet.

Text continues on P.86.

Table 11. The salinity of stations along the Trent and Mersey Canal system between December 1971 and December 1972.

Station No.	No. of Samples.	Mean salinity (mgl^{-1} Cl)	± standard deviation (mgl^{-1} Cl)	Minimum (mgl^{-1} Cl)	Maximum (mgl^{-1} Cl)
1	11	69	55-85	45	92
2	13	64	48-80	44	94
3	5	61	39-83	37	89
4	4	67	43-91	39	91
5	13	74	52-99	33	113
6	12	4640	3700-5580	2990	5920
7	8	4990	4500-5480	4270	5770
8	13	4090	2350-5820	4760	6290
9	13	3570	2630-4520	2020	5750
10	10	3370	2550-4180	2790	5470
11	11	2870	1790-3960	2040	5230
12	5	3290	2240-4340	2370	5040
13	6	2700	965-4430	1890	6050
14	7	2590	1100-4080	1340	5770
15	2	2024	-	870	3180

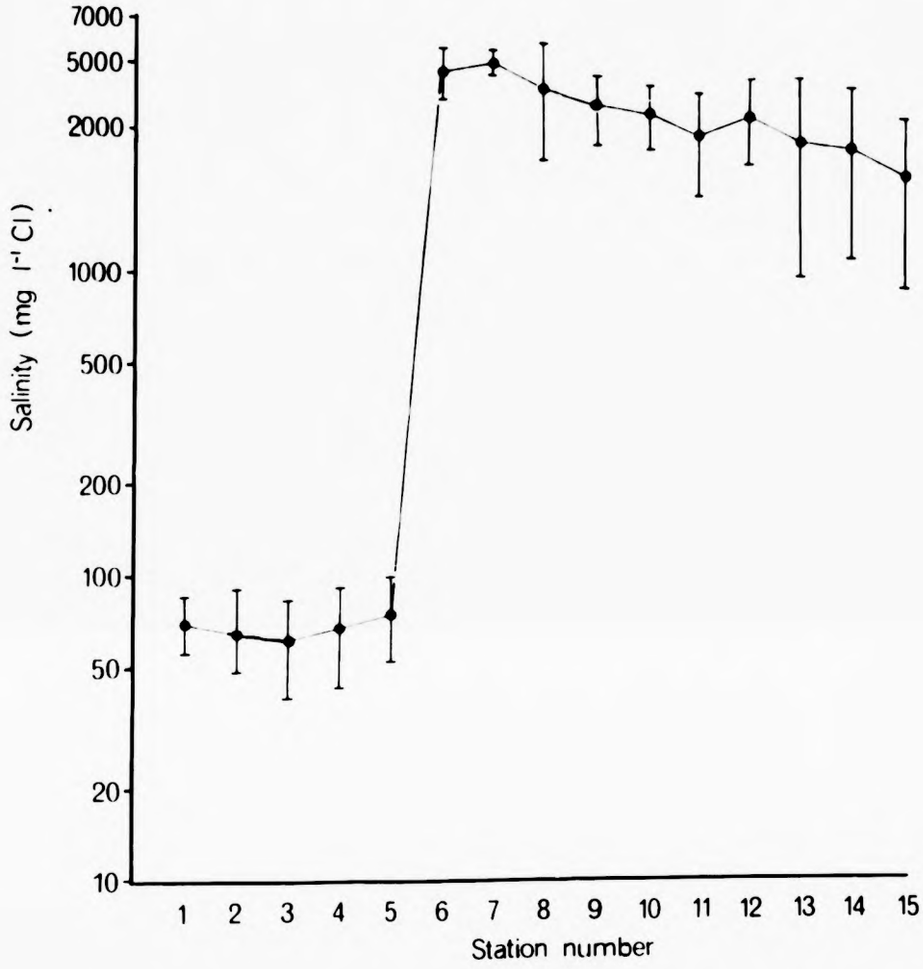


Fig. 12. Mean salinity (± standard deviation) at stations on the Trent and Mersey Canal system

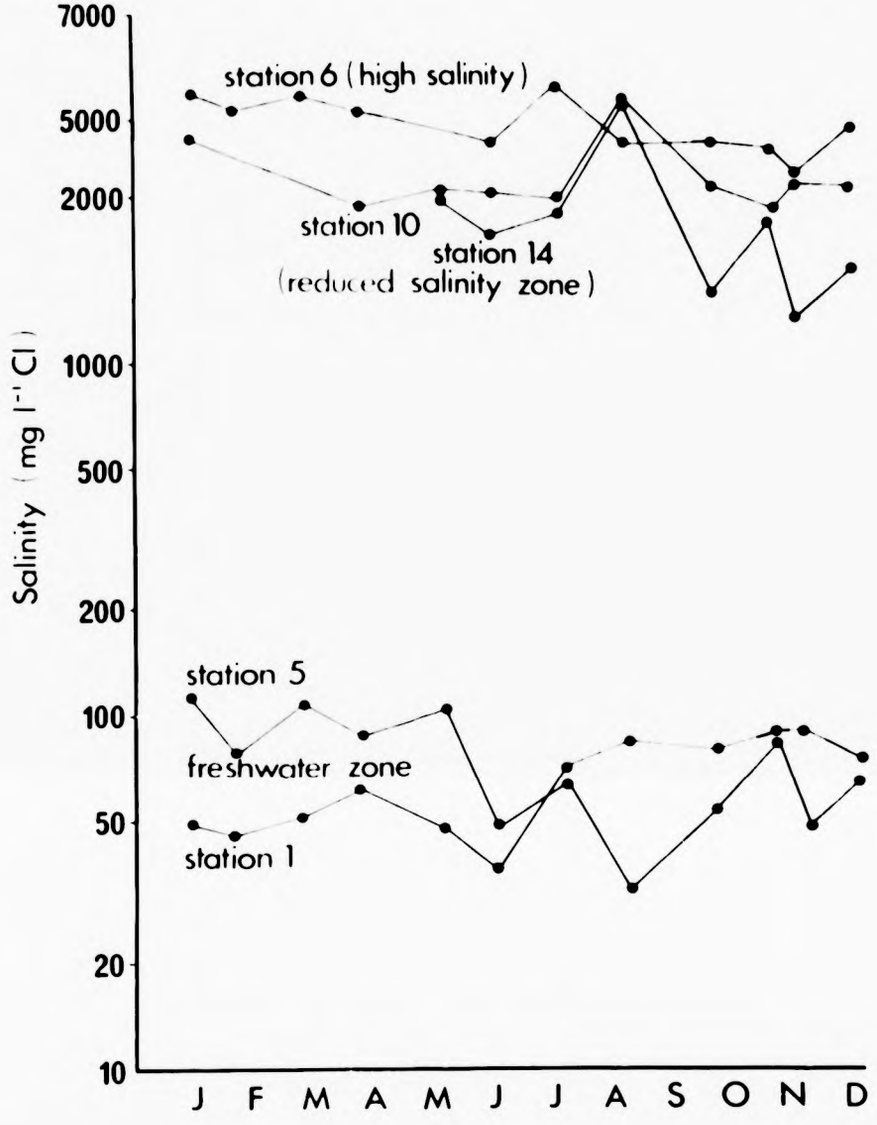


Fig. 13. Seasonal variation of salinity in the Trent and Mersey Canal system , 1972.

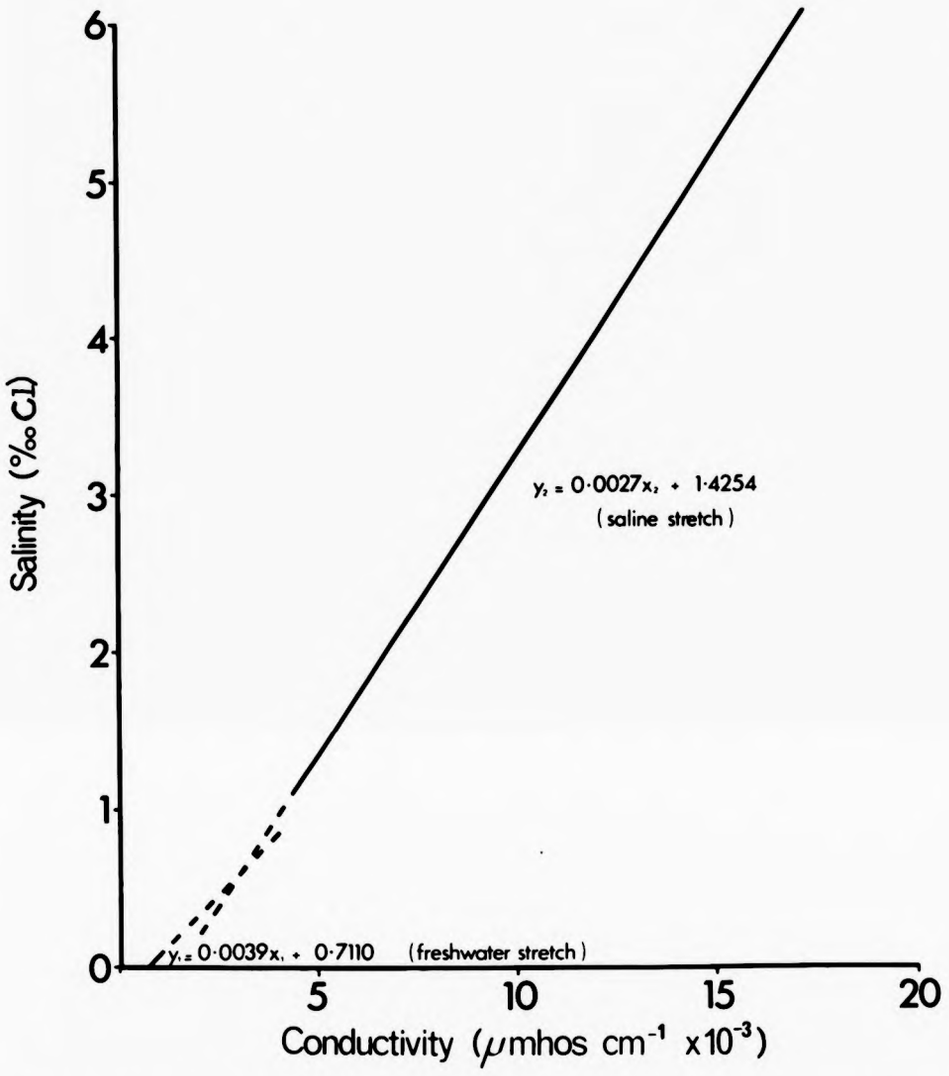


Fig. 14. The relationship between conductivity (y) and salinity (x) in the Trent & Mersey Canal

Table 12. pH at stations along the Trent and Mersey canal system between December 1971 and December 1972.

Date	24.11.71	17. 1.72	7. 2.72	9. 3.72	6. 4.72	18. 5.72	15. 6.72	18. 7.72	17. 8.72	29. 9.72	31.10.72	14.11.72	8.12.72
Station													
1	9.0	7.7	7.6	8.8	8.8			9.2		8.4	7.5	7.8	7.7
2	7.6	7.6	7.2	7.6	8.1	7.8	7.7	7.6	7.5	7.7	7.3	7.7	7.6
3				8.0		8.1	7.9	8.2	7.8				
4				8.0		8.0		8.3	7.8				
5	7.8	7.8	7.8	8.1	8.3	8.3	8.2	8.3	7.8	7.8	7.4	7.7	7.8
6	8.6	9.0	8.7		9.1	8.8	8.6			8.8	8.6		9.0
7	8.5	8.8		9.0				8.9	8.6	8.8	8.4	8.7	8.7
8	8.4	8.7	8.5	9.0	8.7	8.8	8.9	9.0	8.4	8.5	8.2	8.5	8.9
9	8.3	8.4	8.5	8.9	8.8	8.8	8.9	9.0	8.4	8.5	8.2	8.4	8.7
10			8.2		8.7	8.8	9.0	9.3	8.4	8.2	8.2	8.4	8.5
11				8.5	9.2	9.4	9.3	9.7	8.9	8.6	8.1	7.5	7.3
12								9.7	9.2		8.0	8.0	7.9
13								9.8	8.6	8.4	8.1	8.1	8.0
14							8.8	9.7	8.5	8.4	8.2	7.7	7.7
15						9.1							

Table 13. Dissolved oxygen in the Trent and Mersey canal system on 18.5.72.

Station number.	Dissolved oxygen mg l^{-1}	Dissolved oxygen %saturation	Approximate time (B.S.T)	Temperature ^{o}C
2	7.4	71	09.10	12.4
3	7.6	72	09.50	12.4
4	7.4	71	10.20	12.4
5	10.2	101	11.00	13.4
6	6.7	70	11.30	16.7
8	8.8	86	12.00	15.4
9	8.0	82	13.00	15.3
10	13.9	143	14.00	14.8
15	11.8	121	15.00	14.8

Table 14. Major ions of the Trent and Mersey canal system (mgl^{-1})

Station No.	24.11.71					18.7.72	
	Ca	Mg	K	Na	Cl	Ca	Mg
1	56	7	14	51	68	66	12
2	85	20	10	110	44	127	32
3	-	-	-	-	-	107	28
4	-	-	-	-	-	107	28
5	86	42	12	145	77	101	32
6	92	20	29	3355	4757	-	-
7	82	17	29	3900	5156	84	23
8	85	19	32	4025	5825	86	29
9	80	18	26	2600	3591	83	20
10	-	-	-	-	-	78	20
11	-	-	-	-	-	71	20
12	-	-	-	-	-	83	22
13	-	-	-	-	-	79	22
14	-	-	-	-	-	81	21

Table 15. Chemical analysis of samples taken from the Trent and Mersey Canal between stations 12 and 13 by ICI Ltd., August 1972.

	mgl^{-1}
Ca	60-82
Mg	20-24
Sr	0.29-0.35
Al	0.2-0.4
Fe	0.18-0.55
Mn	0.13-0.28
Cu	0.01- <0.02
Zn	0.01-0.06
V	0.01-0.026
Mo	0.002-0.003
Cr	0.001-0.002
Pb	0.005-0.011
Ni	0.007-0.015
NH_3	0.3
NO_3	15
NO_2	0.2

Physical Factors.

Temperature (table 16).

Minimum temperatures were in the region of 3.4°C, and thin ice was only seen on one occasion at some of the freshwater and low salinity stations. The brine in the saline stretches was calculated to cause a freezing point depression of around 0.5°C and this may have been sufficient to reduce the amount of freezing in the mild winter of 1972. Summer temperatures reached between 12-17°C though the canal at station 6 was regularly warmer than at other stations. This elevated temperature may have been due to the temperature of the brine effluent.

Flow.

Mean rates of flow were provided by British Waterways Board. On the Trent and Mersey Canal above the junction with the Middlewich Link Canal, mean flow was 10.5 megaliters per day, and below the junction of the canals it was 14 megalitres per day.

Suspended solids.

North West Water Authority provided information that clay is dredged for use in canal maintenance just below station 5 and that suspended solids are intermittently high for some distances downsteam. It also reports that there are no discharges of organic matter into the canal and that levels of organic matter are low.

Biological components.

The flora.

No macrophytes were found in those parts of the canal system studied. Filamentous algae, predominantly Cladophora sp. were common at station 1-5. Mosses and liverworts were present at stations 1-3. Enteromorpha intestinalis was abundant along the water line at stations 8-15 though limited at stations 6 and 7, and a high green-brown turbidity, particularly at station 1, indicated the presence of microscopic algae.

Table 16. Temperature of samples taken from the Trent and Mersey canal system during 1972.

Date	17. 1.72	7. 2.72	9. 3.72	18. 5.72	15. 6.72	31.10.72	14.11.72	8.12.72
Station Number	Temperature (^{o}C)							
1	4.5	3.0	6.0			10.5	5.0	3.0
2	4.0	5.0	5.0	12.5	16.0	11.0	5.0	4.0
3			5.0	12.5	16.0			
4			5.0	12.4				
5	4.5	5.0	5.5	13.4	8.2	11.0	5.0	4.2
6	5.0	5.0		16.7	8.6	13.5	8.0	8.2
7	4.5		8.5			13.0	7.0	5.2
8	4.5	5.0	5.5	15.4	8.9	11.5	6.5	5.5
9	4.0	5.0	10.0	15.3	8.9	11.0	6.5	5.5
10				15.2	9.0	11.0	6.0	5.5
11			5.0	14.9	9.3	11.0	5.7	3.0
12						11.0	5.0	5.0
13						11.0	5.0	4.5
14					8.8	11.0	4.0	4.5
15				14.9				
Mean temp.	4.4	4.6	6.2	14.4	10.4	11.4	5.8	4.8

The fauna.

Eighty-five distinct species were recorded in the canals, and only nine of these were absent from the freshwater stations (1-5). A complete species list is presented in table 17 where abundance of each species, at every station, is presented. Oligochaetae, Porifera and Bryozoa are only recorded as present (x) or absent because of the difficulties of quantification. Stations have been ranked from low to high salinity for the presentation of data. Abundance of the more adequately represented groups at the various salinities is presented in fig.15 and the frequencies of the major Crustacean species are presented separately in fig.16 where they are expressed in terms of relative abundance. This minimises error arising from different numbers of samples having been taken from various stations. Because the co-existence of three species of the same genus (Gammarus) is exceptional, the seasonal variation in abundance was examined and is presented in fig.17. Kendall's tau (Kendall 1962) was computed for each combination to measure the degree of association (appendix 3). The distribution of Gammarus duebeni showed no correlation with those of the other two Gammarus spp. but there was a strong negative correlation between the distributions of G.tigrinus and G.zaddachi ($p < 0.02$).

Several types of frequency distributions could be identified for the other invertebrates, and they could be categorised according to the range and the skewness of their distributions. A mode at stations 1-5 was taken to indicate a limnobiont or euryhaline group, (the degree of tolerance being apparent from the range) and a mode between stations 6-15 indicated some degree of preference for salinity. A number of species were categorised with reference to their established ranges; Gammarus duebeni, G.tigrinus and G.zaddachi are normally estuarine in distribution and in physiological terms are genuine brackish water species (Sutcliffe 1968) and Nais elinguis, Ephydra riparia and Nemotelus notatus are known to be holeuryhaline in range (Carpenter 1928; Burke 1942b, Remmert 1955a,b; Remane and Schlieper 1958). Major

Text continues on P.100.

Table 17, Species found in the Trent and Mersey and Middlewich Link Canals.

		1	2	3	4	5	15	14	13	12	11	10	9	8	7	6
Porifera																
Desmospongiae	unidentified	x	x													
Platyhelminthes																
Tricladida	Dendrocoelum lacteum (Muller)			1		2	1	1								
Annelida																
Oligochaeta	Nais elinguis Muller		x		x		x	x		x		x	x	x		
	Tubifex tubifex (Muller)		x		x								x	x		
	Limnodrilus cervix Brinkhurst	x														
	Limnodrilus claparediamus Ratzel	x	x			x							x			
	Limnodrilus hoffmeisteri Claparede	x	x										x			
	Eiseniella tetraedra Sav.			1												
	unidentified	x	x		x	x	x	x		x		x	x	x		
Hirudinea	Helobdella stagnalis (Linnaeus)	1														
	Erpobdella octoculata (Linnaeus)	5			1											
Bryozoa	unidentified	x														
Mollusca																
Gastropoda	Potamopyrgus jenkinsi (Smith)	1	1	4½th	21th	20th								1		2
	Bithynia tentaculata (Linnaeus)	1	1	46		2		1								
	Viviparus viviparus (Linnaeus)	120														
	Limnaea (Limnaea) stagnalis (Linnaeus)		1	1		1										
	Limnaea (Radix) auricularia (Linnaeus)		1													
	Limnaea (Radix) pereger (Muller)	4	49	1	2	20										
Lamellibranchiata	Anodonta cygnaea (Linnaeus)	3														
	Sphaerium (S.) rivicola (Lamarck)	5														
	Sphaerium (S.) corneum (Linnaeus)				3											
	Sphaerium (Musculium) transversum (Say)	1		7												
Crustacea																
Amphipoda	Crangonyx pseudogracilis Bousfield	8	152	8	43											
	Gammarus duebeni Lilljeborg			3	11	36	3	137	32	6	25	9	9	1		
	Gammarus tigrinus Sexton						44	889	29	1	1	1	19			
	Gammarus zaddachi Sexton						3	8	32	62	172	222	9			
Isopoda	Asellus aquaticus Linnaeus	58	834	48	64	273	13	7	148	28						

th =thousand x = species present

		1	2	3	4	5	15	14	13	12	11	10	9	8	7	6
Insecta																
Collembola	unidentified.	3												1		
Odonata	Ischnura elegans (van der Linden)		8	2	6	2										
Hemiptera	Callicorixa praeusta (Fieber)		1													
	Corixa affinis Leach				1											
	Sigara stagnalis (Leach)					1										
	Sigara dorsalis (Leach)					2								1		
	Sigara distincta (Fieber)										1					
	Sigara fossarum (Leach)					1										
Neuroptera	Sialis lutaria (Linnaeus)	1														
Trichoptera	Phryganea grandis (Linnaeus)	1														
	Phryganea obsoleta (Hagen)		3		3											
	Phryganea varia (Fabricius)				2											
	Limnephilus flavicornis (Fabricius)			1												
	Anabolia nervosa (Curtis)	1														
	Polycentropus flavomaculatus (Pictet)	2														
	Holocentropus picicornis Stephens	3														
	Holocentropus stagnalis (Albarda)			1												
	Ecnomus tenellus (Rambur)	1														
	Hydroptila sp.					17										
	Athripsodes aterrimus (Stephens)		7	36	1											
Coleoptera	Haliplus fluviatilis Aubé			9	2	2										
	Haliplus immaculatus Gerhardt				6											
	Haliplus lineatocollis (Marsham)				9											
	Haliplus wehnckei Gerhardt				14	2										
	Haliplus sp. (unidentified)		1								1			16		
	Stictotarsus duodecimpustulatus (Fabricius)			1												
	Hydroporous palustris (Linnaeus)											1				
	Potamonectes depressus (Fabricius)			11	1	11										
	Laccophilus hyalinus (Degeer)	2	2													
	Noterus clavicornis (Degeer)	1	1													
	Helophorous brevipalpis Bedel	1						1				1			1	
	unidentified larvae	2	2	1	1	9			1			1			1	

		Station number														
		1	2	3	4	5	15	14	13	12	11	10	9	8	7	6
Insecta																
Diptera																
(Tipulidae)	unidentified					1										
(Ceratopogonidae)	Dashyhelea sp.	2											1			
	other Ceratopogonidae						3					2		1	1	
(Chironomidae)	Pentaneura sp.		2	2												
	Macropelopia sp.													1		
	Procladius sagittalis (Kieffer)	7	67		1	16			1	1		23	218	187	357	9
	Cricotopus sylvestris (Fabricius)		34	2	1	1		7	4		34	96	877	419	126	
	Cricotopus trifasciatus (Panzer)	19														
	Eukiefferiella brevicalcar (Kieffer)		1													
	Prodiamesa olivacea (Meigen)	1														
	Limnophyes sp.										1		10			2
	Metriocnemus atratulus (Zetterstedt)								2			41	7			
	Chironomus riparius Meigen	23	1			10		37	218	1	249	138	148	28	10	
	Chironomus annularius (Degeer)		7						8		53	1				
	Chironomus plumosus (Linnaeus)		19			3		16	10		1	1				
	Chironomus cingulatus Meigen		2			1	1					2	8	4		
	Einfeldia sp.	43	186		4	5	2	85	8	3	51	4				7
	Cryptochironomus sp. 'defectus' group	22	6													
	Cryptochironomus sp.'pararostriatus' group	102				2										
	Cryptochironomus sens. lat.									1			14			
	Glyptotendipes f. glaucus Meigen	28	120	3	21		7	21	4		101	8	12	6	28	1
	Limnochironomus sp.		2		6					2				1		
	Polypedilum laetum (Meigen)				2											
	Polypedilum nubeculosum (Meigen)	3	105													
	Polypedilum convictum (Walker)	1														
	Pentapedilum sp.	4	2													
	Microtendipes sp.	2	41						1		1					
(Stratiomyidae)	Nemotelus notatus Zetterstedt							1				3	2			
(Ephydridae)	Ephydra riparia Fallen											1	7	8	1	

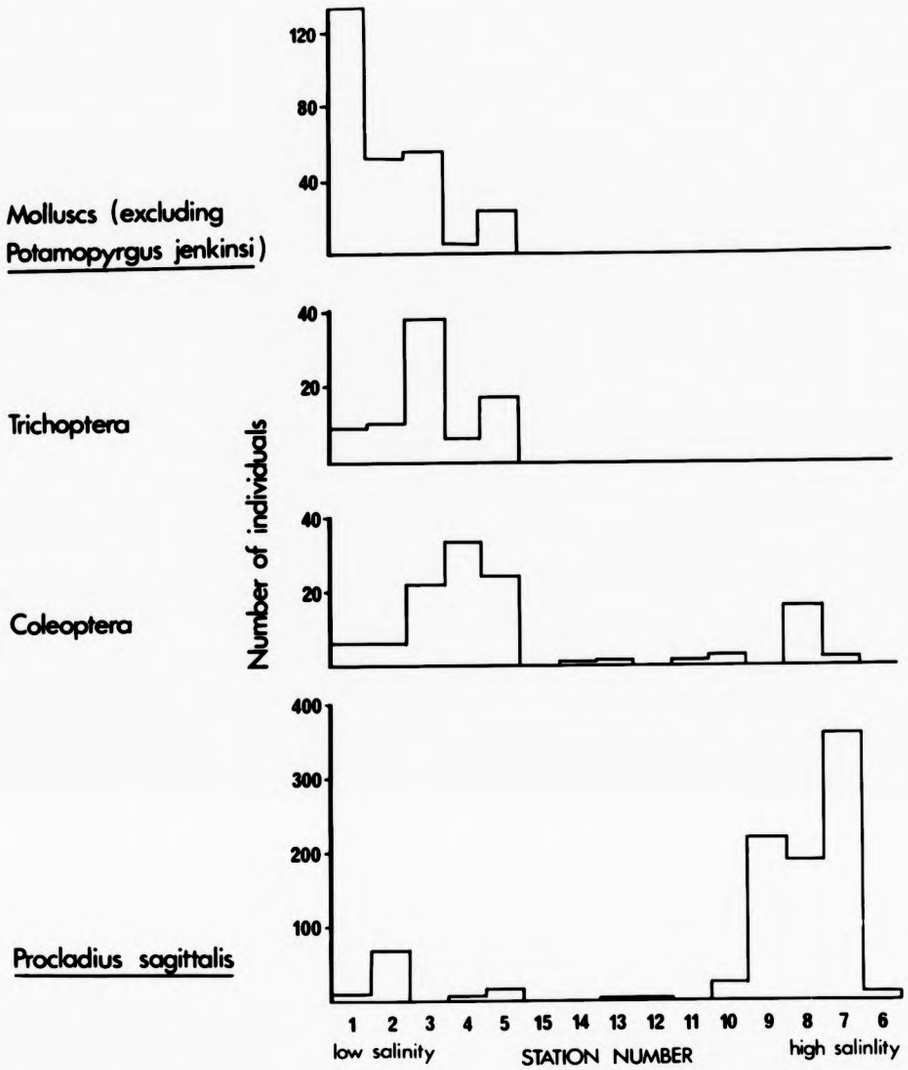


Fig. 15a. Cumulative frequency of invertebrates taken from the Trent & Mersey canal system.

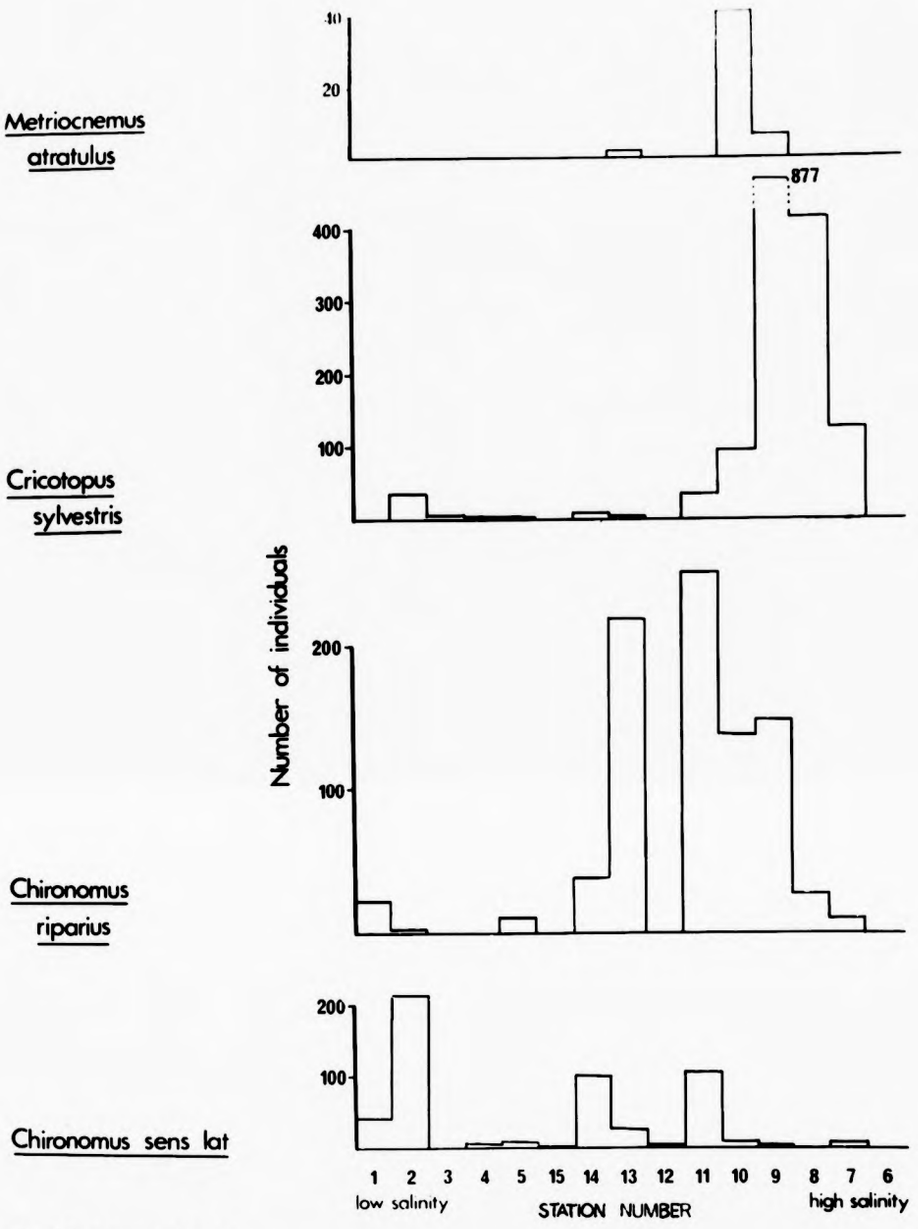


Fig. 15 continued, 15b.

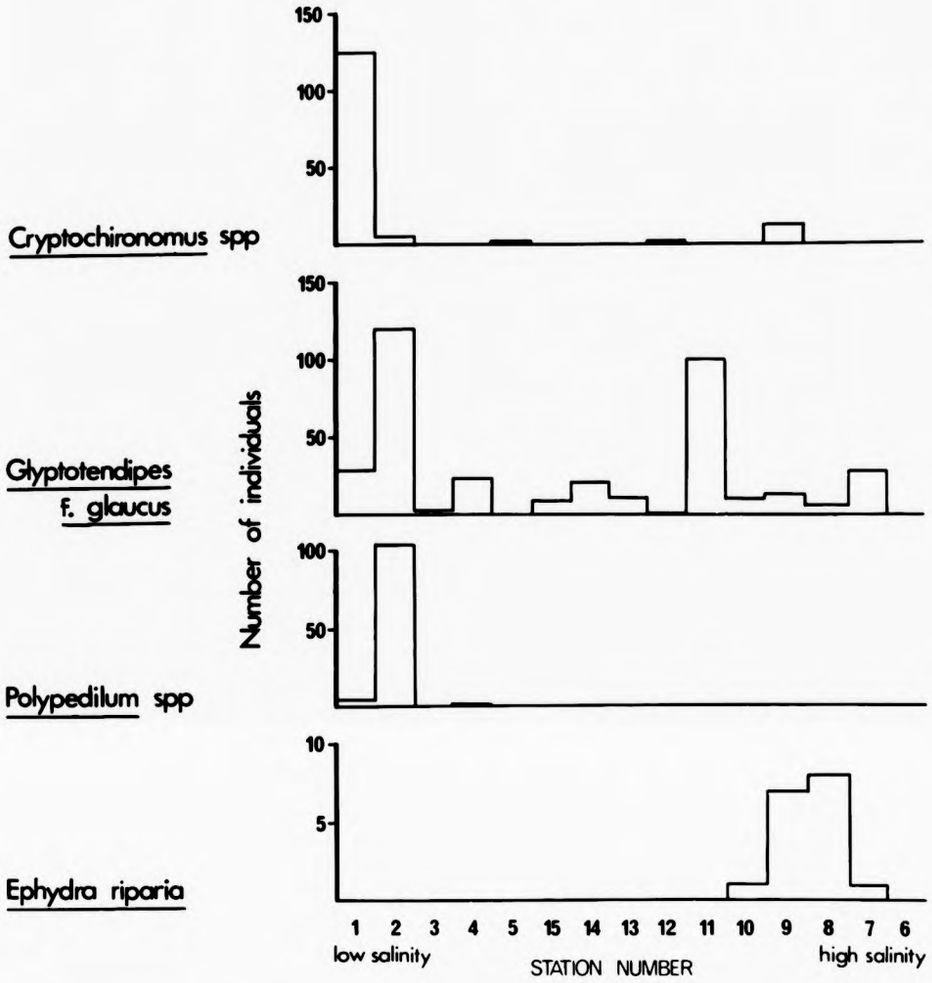


Fig. 15 continued, 15c.

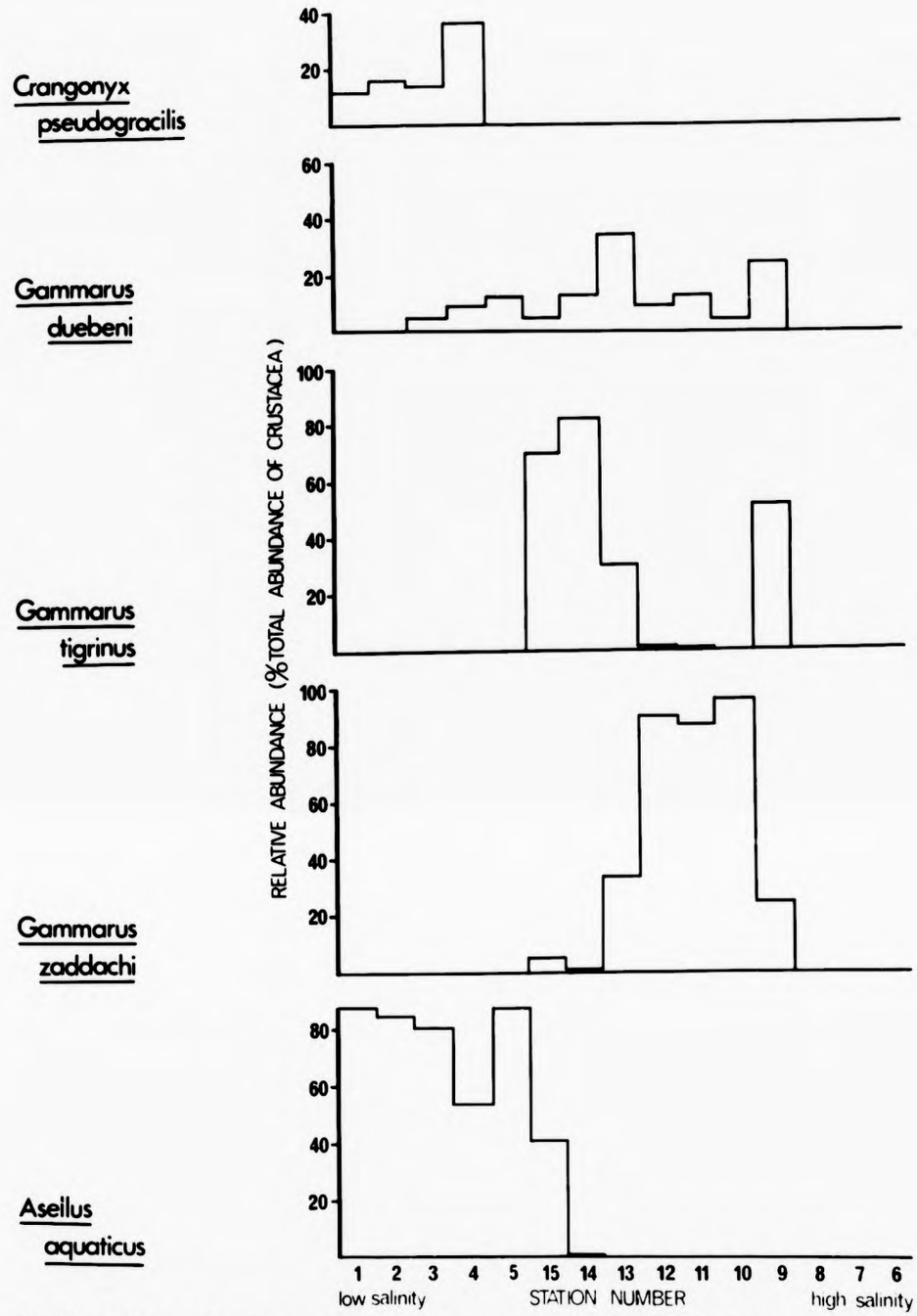


Fig. 16. Relative abundance of Crustacea along the Trent & Mersey canal system.

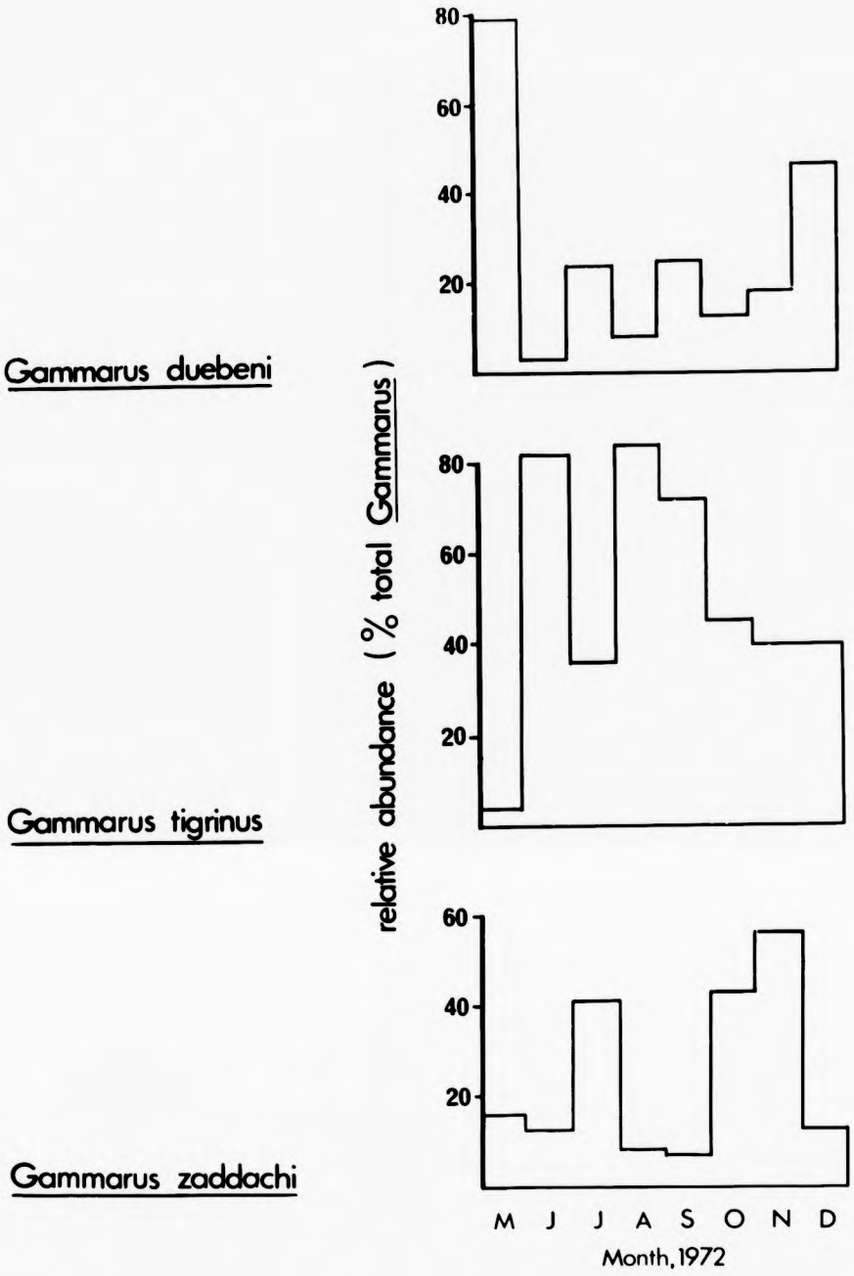


Fig. 17. Seasonal variation in the relative abundance of three species of Gammarus in the Trent & Mersey Canal.

Table 18. Degree of tolerance of various categories of invertebrates in the Trent and Mersey canal system.

Category	Stations at which recorded.	Range of mean salinity (mgl^{-1})
Stenohaline limnobionts (and possibly euryhaline to the 1st degree). -all the Trichoptera present -all the Mollusca present (with the possible exception of Potamopyrgus jenkinsi) -Crangonyx pseudogracilis -Polypedilum spp. -Ischnura elegans -all the Hirudinea present	1-5	Freshwater to 100 mgl^{-1} Cl (168 mgl^{-1}S)
Euryhaline limnobionts of the second degree. -Asellus aquaticus -Dendrocoelum lacteum	1-5 14-15	Freshwater to 2600 mgl^{-1}Cl (4370 mgl^{-1}S)
Euryhaline limnobionts of the third degree. -Chironomus spp.* (excluding C.riparius). -Cryptochironomus spp. -Glyptotendipes f. glaucus -Tubifex tubifex -Limnodrilus spp.	1-5 15-8	Freshwater to 4100 mgl^{-1}Cl (6890 mgl^{-1}S)

Category	Stations at which recorded.	Range of mean salinity (mgl^{-1}).
Halophye organisms of the first degree. -Metriocnemus atratulus -Chronomus riparius -Potamopyrgus jenkinsi	maximum at 10-13.	2700-3400 mgl^{-1}Cl (4540-5710 mgl^{-1}S)
Halophye organisms of the second degree. -Procladius sagittalis -Cricotopus sylvestris -Limnophyes sp.	maximum at 7-9	3500 - 5000 mgl^{-1}Cl (5890 - 8400 mgl^{-1}S).
Genuine Brackish Water organisms. -Gammarus duebeni -G.tigrinus -G.zaddachi		
Holeuryhaline ** -Nemotelus notatus -Ephydra riparia -Nais elinguis	All stations.	
Largely independent of salinity. (though with clear preference for fresh water). -all Coleoptera present -all Hemiptera present		

* some of these may be halophye

**according to established ecology of the species.

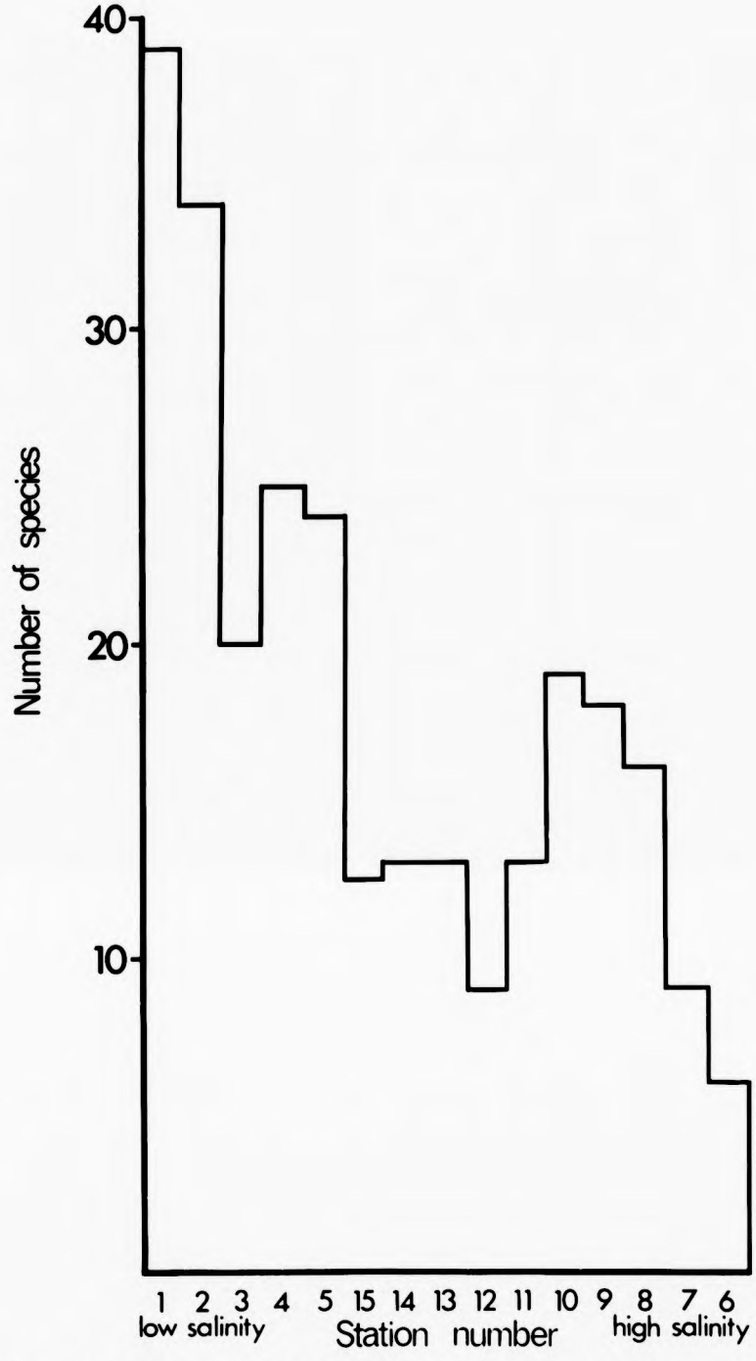


Fig. 18. Number of species at stations along the Trent and Mersey canal system

groups could be allocated accordingly to those categories of tolerance used in the previous chapter (table 18). As the salinity gradient was discontinuous and low salinities were not represented, euryhaline 1^{o} species and species which are only slightly halophye may have been included in group 1. The range of the mean salinity for each of these groups is in accordance with that found for the respective groups in the Crow Brook study and thus confirms that the allocation to categories was not entirely arbitary.

Numbers of species taken at each station are presented graphically in fig.18 and a fall off in diversity with increasing salinity is apparent. A small peak in the diversity at stations 8-11 is probably due to a greater sampling intensity at these sites (table 10,p75). The index of diversity (α) makes allowance for the effect of the latter on species diversity and has been calculated for each station in table 24(p125). As the R.Dane passes under the Trent and Mersey canal close to station 10, some recruitment of species, especially those with aerial stages, may have occurred along this stretch. Total numbers of individuals taken from each station are also presented in table 24 and it is apparent that there is initially an abrupt decline where the effluent is most concentrated, though numbers expand dramatically as the canal becomes diluted.

DISCUSSION

1. The general water quality of the canal.

A low number of species at stations 6 and 7 combined with low absolute numbers (table 24,p125)indicates that conditions along this stretch are more severe than would be expected as a result of the brine effluent alone, and it is thought that both high suspended solids and discharges containing iron oxide affect this stretch. Therefore, although the presence of a species implies its tolerance of salinity, the absence of a species cannot be taken to imply a lack of tolerance to salinity. A

rich fauna at station 9 indicates that favourable conditions are restored by that point, probably as a result of the contribution of water from the Middlewich Link Canal. The rest of the canal system is apparently not polluted by effluents other than brine and Holland (1976b) confirms that no other pollutants are known to the North West Water Authority. Although exceptionally high pH values were recorded, a well balanced fauna in the freshwater stretches testifies that these are not particularly disadvantageous. High daytime pH values are not an uncommon feature in slow flowing waters where the photosynthetic action of both macrophytes and algae, facilitates the precipitation of calcium carbonate by removing carbon dioxide from the water (Hynes 1970). The buffering capacity of the water can similarly be lost where the removal of carbon dioxide causes the oxidization of ferrous bicarbonate to ferric hydroxide, a process which is promoted in conditions of supersaturation with oxygen. Supersaturation combined with high pH values was detected at stations 5, 10 and 15 on 18.5.72 and high pH values throughout the canal system, particularly in summer, were undoubtedly photosynthetically mediated.

2. The effects of the brine on the ecology of the canal.

Unlike Crow Brook, the salinity gradient in the canal was not continuous and increasing, but was seen as an abrupt transition from freshwater to high salinity (circa $5^{o}/_{oo}Cl$). The effect was the total elimination of the majority of species immediately beyond the freshwater limit of both the Trent and Mersey and the Middlewich Link Canal, as well as a decline in total numbers. A replacement fauna with a different species composition succeeded in the less extreme stretches and some recovery of the original fauna occurred with increasing downstream dilution. The changes in the fauna are discussed in terms of individual species tolerances, numbers of species and the species - numbers relationship.

(a) Individual species tolerances.

When the distributions of various species in the Trent and Mersey Canal are considered, it becomes apparent that there are discrepancies between these and the established tolerances of

the species. The distributions of the various groups are discussed in taxonomic sequence, and an explanation for the discrepancies is sought at the end of the section.

PORIFERA AND BRYOZOA were not identified and were only taken occasionally in the freshwater stretches.

PLATYHELMINTHES. Dendrocoelum lacteum was the only species of flatworm found in the canal and was taken not only from freshwater stations but also from the most downstream stations of the saline stretch (14 and 15). The species is quite strongly euryhaline in the Baltic, extending in places to salinities of 7.8°/oo S (Forsman 1956). In the laboratory Gresens (1928) demonstrated that freshwater D.lacteum could tolerate up to 7°/ooS and that this was also the limit of reproduction. With gradual acclimation he succeeded in demonstrating a tolerance of 15°/ooS.

OLIGOCHAETA. Of the six species identified in the canal only Limnodrilus cervix and Eiseniella tetraedra were uncommon, both being recorded on one occasion and only in the freshwater stretches. No oligochaetes were found at the two most saline stations (6 and 7) even though Nais elinguis is known to inhabit hypersaline waters (Remane and Schlieper 1958) and Tubifex tubifex, Limnodrilus claparedeanus and L.hoffmeisteri are all considered markedly euryhaline in the Baltic where they are found in the zone 8°/ooS - 16°/ooS (Remane & Schlieper, 1958). The presence of the same three tubificids in Ijsselmeer since 1940 (Beaufort 1953) further confirms their tolerance of moderate salinities and it would seem that their absence from the saline stretches of the canal may confirm the presence of other pollutants along this stretch of the canal.

HIRUDINEA. Only seven specimens were taken, representing the species Erpobdella octoculata and Helobdella stagnalis. It is not surprising that neither was found outside the freshwater

stretches as even the most dilute parts of the saline stretch approach the upper salinity limit of the species. Rawson and Moore (1944) found that although H.stagnalis and a species E.punctata (Leidy) were present in the saline lakes of Saskatchewan up to salinities of 2,250 $mgl^{-1}S$, their abundance declined above 770 $mgl^{-1}S$. Pyefinch (1937) failed to find H.stagnalis in any of the Bardsey pools with salinities greater than 400 $mgl^{-1}Cl$ (circa 740 $mgl^{-1}S$) and Whitehead (1935) pointed out that the distribution of H.stagnalis and E.octoculata correlates with the presence of food, and as the former feeds on gastropods it is hardly surprising that this species at least, is absent outside the freshwater stretches of the Trent and Mersey Canal.

MOLLUSCA (fig.15a). Six species of Gastropoda and four bivalve species were found in the canal and all specimens were taken from the freshwater zone with the exception of three specimens of Potamopyrgus jenkinsi. The latter was found in myriad proportions at stations 3-5 though an explanation of the phenomenon is not apparent, chloride levels being virtually constant (appendix 2), unless it relates to possibly higher levels of sodium in that stretch of the canal. It is significant that Holland (1976b) mentions historic discharges of brine upstream of the present discharge. It seems probable that the three specimens found in the high salinity stretch were drift individuals as the snail can utilize surface tension between its operculum and the surface (personal observation) and could travel thus, with the flow. If the species was indeed tolerant of the high salinity zone it would be expected to occur there in far greater numbers, as a result of recruitment from the nearby prolific population. The distribution in the canal supports the suggestion of Bondesen and Kaiser (1949),that having developed an osmoregulatory system which permitted it to invade freshwater, the snail is now incapable of maintaining itself at high salinities.

As the Mollusca have attracted the interest of research workers, their ranges in relation to salinity are well documented, (Johannsen1918, Schlesch 1937, Lindberg 1948, Jaeckel 1950). Remane and Schlieper (1958) report that 70% of the Northern and Central European species are probably stenohaline limnobionts, though a few species have been found locally at salinities of up to 2°/ooS. Anodonta piscinalis Nilsson has been found at about 4°/ooS at Schlei (Jaeckel 1950), and is exceptional for its genus. Freshwater molluscs, and particularly bivalves, are inevitably susceptible to high salinities owing to their high permeability. Some forms, however, can tolerate brackish water, and despite wide variations in their blood concentration, their cells can adjust by modification of their solute content (Lockwood 1963). This is apparently the case in most of the Pulmonata and all of the Prosobranchia which display various degrees of eury-halinity in the Baltic.

North European limits of the species found in the Trent and Mersey canal are listed in table 19. (after Remane and Schlieper, 1958), and it seems apparent that the range of the species in the canal is not necessarily limited by salinity, per se. Even the lowest of these figures from Randersfjord would allow that B.tentaculata and L.pereger could tolerate the saline stretches of the canal.

Table 19.
Maximum tolerance of some molluscs for salinity in a number of Baltic habitats.

	Salinity (°/ooS)				
	JOHANNSEN	JAECKEL		SCHLESCH	LINDBERG
	Randersfjord	Schlei	Baltic	Baltic	Baltic
Bithynia tentaculata	3-4(5)	6	6-7	7	7
Viviparus viviparus	1-3			3	
Limnaea stagnalis	1.5	6	7	7	7
L.auricularia	2	6	1.5	1.5	
L.ovata (= Radix pereger).	6	13.7	10-11	10	

Tolerances of salinity in ecological situations outside the Baltic are in most cases lower, though Remane and Schlieper (1958) mention that B.tentaculata was recorded at 12°/ooS in the Zuiderzee. Many dead specimens were found there, and Beaufort (1953) interpreted these as being specimens carried by the Ijssel into the Zuiderzee and took them as evidence that the species had not succeeded in establishing itself. Beaufort documents the recruitment of freshwater molluscs to Ijsselmeer, and reports the appearance of 19 species between 1932 and 1943 as salinity fell from around 7°/ooS to 0.3°/ooS. These included Anodonta piscinalis, Pisidium spp., Sphaerium spp. and Lymnaea stagnalis. Rawson and Moore(1944) found 26 species of gastropod in the saline lakes of Saskatchewan and these were common up to 2,250 mgl^{-1}S with L.stagnalis being dominant. Abundance declined in the more saline lakes and they concluded that salinity became harmful to gastropods at around 3,000 mgl^{-1}S. They report a suggestion that this is attributable to direct physiological factors rather than ecological factors. Sphaeriidae and Pisidium spp. were more sensitive to salinity and rarely found above 2,550 mgl^{-1}S. It seems therefore, that Baltic distributions of the molluscs may be exceptional.

CRUSTACEA.

Three mixohaline species of Gammarus occur in the canal, G.duebeni, G.tigrinus and G.zaddachi and the distribution of the former in Britain has already been discussed. G.tigrinus is a North American species first recorded in Britain in 1931 (Sexton and Cooper 1939) and which is restricted to brackish water or freshwaters with high ion content, (Hynes et al. 1960). G.zaddachi is an estuarine species which is generally accepted as occupying the zone immediately below the freshwater outlet in river mouths. (Serventy 1935).

The co-existence of three members of the same genus in the same habitat is somewhat unusual, but a similar situation has arisen in the Netherlands since the alien G.tigrinus arrived there in 1964. Although populations of G.zaddachi and G.duebeni had previously been well established in the dykes, canals and other

brackish waters there, a fresh survey of many of these habitats in 1973 (Smit 1974) showed that both of these indigenous species had been almost totally displaced by G.tigrinus. Very few G.duebeni could be found, and only then in very variable salinities, and G.zaddachi remained only in high salinity areas where it frequently co-existed with G.tigrinus. The evidence from the Trent and Mersey Canal also indicates that competitive interactions are taking place between at least two of the species, G.zaddachi and G.tigrinus, and this relationship will be discussed first.

The strong negative correlation between G.zaddachi and G.tigrinus is significant at the 2% level (Appendix 3) and their distributions show a clear zonation (fig.16,p95)with G.zaddachi occupying the stretch from Middlewich to Northwich. The relative abundance of G.tigrinus starts to increase at station 13 as that of G.zaddachi starts to decline, and G.tigrinus becomes the dominant species in the canal beyond Northwich. Holland (1976b) also studied the Gammarus spp.of the canal during 1972 and like Smit, he concluded that G.zaddachi accepted higher salinities than G.tigrinus. He was, however, unable to demonstrate a clear zonation of species, and found considerable variations in species dominance from survey to survey. This probably resulted from Holland's sampling from old motor tyres in use as fenders, a technique which although producing abundant numbers, showed a high variability between samples. Furthermore, samples of a microhabit would be expected to give a species dominance relevant to that habitat only. My experience was that G.zaddachi was a bottom dweller, G.tigrinus mainly occupied the Enteromorpha intestinalis fringe, and G.duebeni was more catholic, occupying both habitats. It is significant in this context that over all samples G.duebeni represented 13% of the total gammarid fauna in the present study, whereas Holland's data show that it represented 64% of the Gammarus spp. collected by him from the same stretch in the same year. G.duebeni is notorious as a voracious carnivore (Sexton,1924 ; Forsman 1951) and could well be expected to dominate a motor tyre fauna.

If Holland's (1976b) data from separate sampling occasions are grouped to give total distribution for the whole survey, some of the sampling variation is eliminated, and a rather clearer picture emerges (table 20).

Table 20.

Distribution of *Gammarus spp.* in the Trent and Mersey Canal from Holland (1976b) (data from separate sampling occasions are here grouped). d = *G.duebeni*, t = *G.tigrinus*, z = *G.zaddachi*.

Locality	Station No. *	No. *Gammarus* taken.			Relative abundance (% total Gammarus spp)		
		d	t	z	d	t	z
Middlewich	10	110	19	3	83	14.5	2.5
Broken Cross	11/12	128	75	252	28	16.5	55.5
Barnton	13	486	67	53	80	11	9
Acton Bridge	13/14	300	87	78	64	19	18
Dutton	14	565	176	19	74	23	3
Daresbury	15	149	147	1	50	49.5	0.5
TOTAL		1738	571	406	64%	21%	15%

*The station numbers given are those used in the present study where appropriate, intermediate points have been allocated the combined numbers of the nearest upstream and nearest downstream stations.

If the relative abundances of *G.zaddachi* and *G.tigrinus* are then combined with the data from the present study, it confirms the zonation of the two species, with *G.zaddachi* dominating the Middlewich to Northwich stretch (fig.19).

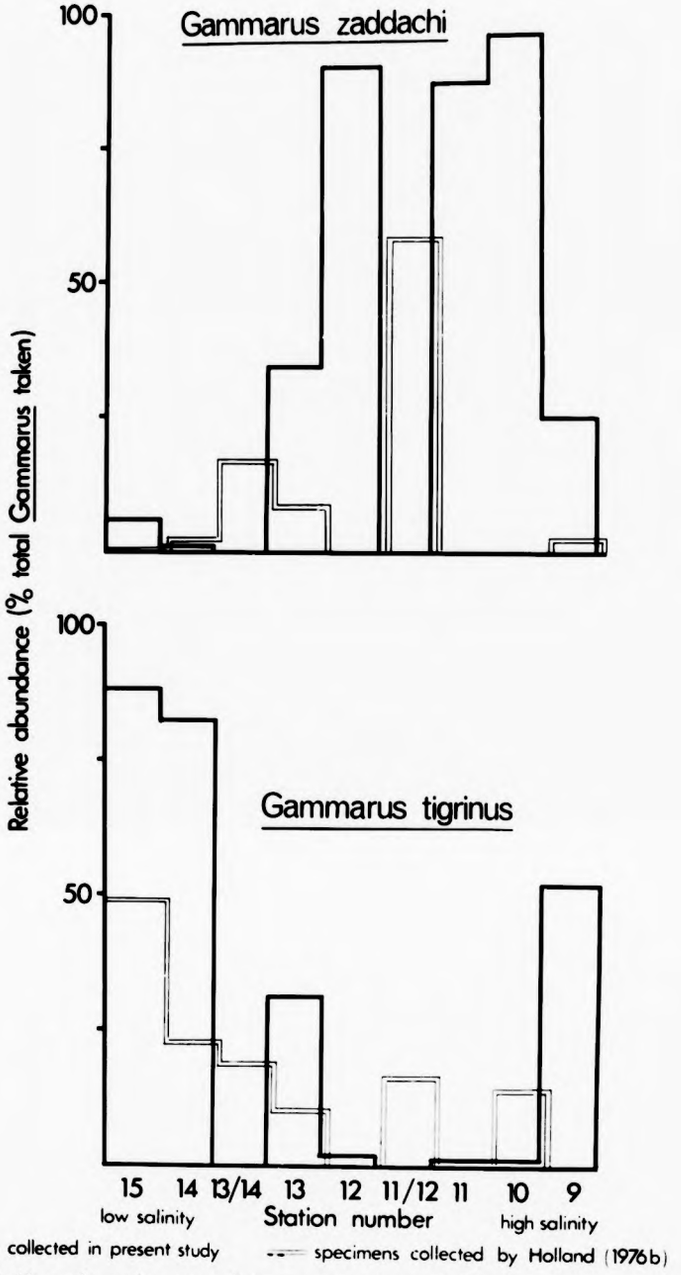


Fig. 19. Relative abundance of two species of Gammarus collected from the Trent & Mersey Canal in two investigations.

In terms of absolute salinities, Holland's conclusion that G.zaddachi accepts higher salinities than G.tigrinus is borne out. Mean salinities at stations 9-12 were all about 3000 mgl^{-1}Cl (5040 mgl^{-1}S) (table 11, p78). Conversely abundance of G.zaddachi was declining and that of G.tigrinus increasing by station 13 as mean salinities dropped to 2,700 mgl^{-1}Cl (4540 mgl^{-1}S). This follows the same pattern then as that seen for the two species in the Netherlands.

G.tigrinus also shows some recovery in abundance at station 9 despite high salinities, and G.duebeni, not G.zaddachi, is the dominant shrimp. It therefore appears that conditions at this station are unfavourable for G.zaddachi, leaving G.tigrinus and G.duebeni to compete for the niche. Although the standard deviation of salinities measured at station 9 during the survey was not exceptional, it must be assumed that large fluxes in salinity occur rather regularly. This station was less than a kilometer from the junction of the Trent and Mersey and Middlewich Link canals and as high salinity water would pass through King's Lock on the Trent and Mersey Canal, and freshwater through Stanthorne lock on the Middlewich Link Canal, it seems unlikely that complete mixing would have occurred by station 9. Although G.zaddachi is known to be very tolerant of wide salinity fluctuations (Serventy 1935, Sexton 1942, Kinne 1954, den Hartog 1964), it has rarely been recorded in such conditions when G.duebeni is present. Hynes (1954) recorded both species at the outlet of the Silver burn on the Isle of Man and found a zonation with G.zaddachi dominant between high neap tide level and high spring tide level,and G.duebeni dominant from there into the mouth of the stream where G.pulex asserted itself. G.zaddachi is also at a physiological disadvantage compared with G.duebeni where low salinities occur, being slightly more permeable to water and salts, and having a slightly higher urine flow rate and a higher sodium loss rate in dilute media (Sutcliffe 1968).

It seems then, that in Britain the distribution of the three species is probably governed by the same factors as in the Netherlands, G.tigrinus is apparently unable to displace G.zaddachi at higher salinities and G.duebeni is more successful where large salinity variations can be expected.

Pinkster (1975) reports laboratory and field experiments which give some insight into the effect of salinity on the relative successes of the shrimps. Experiments using G.tigrinus investigated egg laying and survival of juveniles at various salinities and temperatures. Egg laying and development were normal in salinities between 900-1800 $mgl^{-1}Cl$ (1620-3250 $mgl^{-1}S$) where temperatures were between 10-20°C, and where temperatures were 15-20°C, the successful range was extended into lower salinities (360$mgl^{-1}Cl$, 650 $mgl^{-1}S$). These salinity figures correspond well with the limits of the successful range of G.tigrinus in the Trent and Mersey Canal which extended from somewhere below mean salinities of 2020 $mgl^{-1}Cl$ (3400 $mgl^{-1}S$) and up to mean salinities of between 2700-2870 $mgl^{-1}Cl$. (4540-4820 $mgl^{-1}S$).

Although salinity is obviously an important factor influencing the relative distributions of the three species of Gammarus in the canal, the seasonal distribution of the shrimps indicates that their breeding periods may also govern their relative distributions. Fig.17 shows that G.tigrinus dominated the Gammarus fauna each month from June to September with the exception of July when G.zaddachi was the dominant species, whereas G.duebeni was the most abundant shrimp in December and May. As well as having a high frequency in July, G.zaddachi was also more abundant than the other species in October and November. Although it is clear that G.tigrinus prevails through the summer, more information was required for the other two species in the winter months. The data of Holland (1976b) were examined and revealed that G.duebeni was above average proportions in January and March and below them in the August and October samples. The relative abundance of G.zaddachi was above average in October and January. There is therefore some definite indication of seasonal

displacements of abundance between the three species. Caution must be observed in the interpretation of these results, however, as a peak in the relative abundance of a species can result not only from recruitment of that species, but also from a decline in the other two species. Unfortunately absolute numbers of any species from month to month are not comparable owing to variations in sampling intensity and it is impossible to determine whether changes in proportions originate from recruitment or mortality. Fortunately, previous investigations of reproductive periods of various species of Gammarus contribute to an understanding of the seasonal displacements of their peak abundances.

Pinkster (1975) made field observations of the Gammarus populations in the Netherlands and found that G.tigrinus reproduces in summer when temperatures are high and that the reproductive period extends from March to November. Fecundity of G.tigrinus throughout summer in the Trent and Mersey Canal could therefore be attributable to a summer reproductive period. Pinkster calculated that in the average water temperatures of the Netherlands, a female G.tigrinus that hatched in April would take 6 weeks to mature, and therefore reproduce in mid May and continue to do so every 10-15 days through the summer. In our maritime climate, water temperatures in the Trent and Mersey Canal, although higher than those in the Netherlands between December and March, were lower for the rest of the year, and this is possibly responsible for a somewhat contracted breeding season for G.tigrinus in Britain. Hynes' (1955b) original work on the breeding season of the species took place at Frodsham Marsh, only 8-10 km from the Trent and Mersey Canal and confirms a rather shorter breeding season than in the Netherlands. Although he found that breeding began in early March, he found that the first clutch of eggs did not necessarily succeed. The first young appeared in May and these reached maturity rapidly and were breeding by June and July. The parental generation began to die in late May and most had disappeared by mid July. Breeding continued through August and September and then no breeding occurred through the winter, the females being in a resting state. Thus the abundance of G.tigrinus between June and September in the Trent and Mersey Canal is readily explicable in terms of summer fecundity,

the first young presumably having been taken in the June sample. If the low relative frequency of the species in July is significant, it may be due to the death of the parental generation at a time when the young have yet to mature.

The relative distributions of G.duebeni and G.zaddachi may similarly be influenced by their reproductive seasons. Pinkster (1975) examined the reproductive potential of G.duebeni and G.zaddachi, as well as that of G.tigrinus, in the Netherlands. He found that at salinities of around 4000 mgl^{-1}Cl (7,224 mgl^{-1}S) G.tigrinus & G.Zaddachi bred all year round, but in more dilute waters, around 400 mgl^{-1}Cl (720mgl^{-1}S) breeding only took place from October through until March. He estimated that in favourable salinities G.zaddachi could probably produce the same number of offspring as G.tigrinus because, although the shrimp takes longer to mature than G.tigrinus, it can offset this disadvantage by taking advantage of the winter months for breeding. At low salinities G.zaddachi is restricted to winter for breeding when low temperatures only permit slow development of the eggs, and this presumably explains its failure to compete with G.tigrinus at reduced salinities. In the Trent and Mersey Canal numbers of G.zaddachi appeared to be increasing in October and November and this may signify resumption of breeding in the low salinity stretches.

For G.duebeni, Pinkster calculated that an individual born in the April would not mature until October when egg development would be slow. Eggs would not hatch until the first months of the next year, a pattern which is very similar to the reproductive cycle of G.duebeni in Britain.

Hynes (1954) studied the life-cycle of G.duebeni in the Isle of Man, both in a Port Erin stream and on Gansey beach. At the latter site he found increasing numbers of mature females from the end of December through to March. Young appeared between late March and May, and then breeding declined through the summer, at which time the older females were dying. In the stream, mature

females continued to be present all through the summer though the production of young fell off in July and August and from October to January. In the light of these results, the peak in the relative abundance of G.duebeni in May in the Trent and Mersey Canal is almost certainly due to production of young. The December peak is possibly rather too early to have arisen in the same manner unless it results from September breeding such as Hynes (1954) saw in the Port Erin stream.

In summary, the distribution of G.zaddachi and G.tigrinus in the canal can be explained in terms of reproductive potential in different parts of the salinity spectrum. G.tigrinus is generally successful by virtue of its high fecundity, though being somewhat thermophilic it can only breed at summer temperatures. The displacement of indigenous species by G.tigrinus seems less extensive than in the Netherlands and this may be as a result of our lower summer temperatures. G.zaddachi can apparently match the fecundity of G.tigrinus in salinities above 3,500 $mgl^{-1}S$, but not in more dilute waters. The relationship of G.duebeni to the other two species is not so obvious,though the shrimp is breeding for several months before the breeding season starts for G.tigrinus. There is some evidence that G.duebeni is also more tolerant of salinity fluctuations.

One other amphipod occurred in the canal, Crangonyx pseudogracilis, though this species was restricted to stations 1-4 of the freshwater stretches. The species is North American in origin and was first recorded in 1930 in London and is now common throughout the canal system of the Midlands (Hynes 1955a). The species prefers still water environments (Hynes et al 1960) and although Hynes (1956) thought it probable that the species could tolerate saline water, Holland (1976a) never found it at chloride levels greater than 250-230 $mgl^{-1}Cl$.

The co-existence of G.duebeni with this species at stations 3 and 4 (fig.16) is unusual. C.pseudogracilis is frequently found with

G.pulex and G.tigrinus (Hynes 1955a, Holland 1976a) and appears to have different microhabitat requirements and therefore probably does not compete with any of the Gammarus spp. While C.pseudogracilis is thought to be almost entirely vegetarian (Hynes 1956), Forsman (1951) considers that G.duebeni is omnivorous and Macan (1950) suggests it is the most carnivorous of the freshwater Gammarus spp. It may be significant that G.duebeni was taken only from the three freshwater stations nearest to the brine outlet and that C.pseudogracilis is absent from the most downstream of these. Holland (1976b) mentions that before 1971 salt discharges upstream of the present outlet maintained chloride concentrations at 1000-3000 $mgl^{-1}Cl$ and considers the G.duebeni population at this point to be a declining legacy of these conditions. Even though stations 3 and 4 are upstream of these historic brine effluents, and no elevation of chloride levels was detected along the stretch (Appendix 2) nor any stratification, it is conceivable that enriched ionic composition could sustain a small breeding population of G.duebeni, and the abundance of Potamopyrgus jenkinsi at these stations provides circumstantial evidence for this hypothesis. The shrimp certainly does not show the abundance that would be expected in the absence of its competitors, were its full breeding potential being realised.

The Isopoda were represented in the canal by Asellus aquaticus which was the dominant Crustacean of the freshwater stretches, and which was also found at stations 14 and 15 which have mean salinities just below 2,600 $mgl^{-1}Cl$ (4,370 $mgl^{-1}S$). Although no halophye tendency was detected, the salinity gradient being discontinuous, the range was in accordance with that found in Crow Brook where abundance was declining at a mean salinity of 2,200 $mgl^{-1}Cl$ (3,700 $mgl^{-1}S$) and the species was absent where mean salinities were greater than 3,150 $mgl^{-1}Cl$ (5,290 $mgl^{-1}S$). As the two investigations provide similar figures for the apparent upper salinity limit of the distribution of A.aquaticus, it probably represents a real limit for freshwater Asellus in Britain in natural conditions. The species has however been recorded at $15^{o}/_{oo}S$ in the Baltic (Lagerspetz 1958, Segerstråle 1957) and at

25°/oo in the Westphalian brine pools (Schmidt 1913). Gresens (1928) used A.aquaticus of freshwater origin in laboratory experiments and found that without acclimation 5.25°/ooS was the upper limit for breeding, although individuals could survive 7°/ooS. The data of Lagerspetz (1958) are in approximate agreement, and he established 4.9°/ooS as the upper limit for unacclimatised A.aquaticus of freshwater origin. Ecological restraints would lower the limits in natural conditions as osmotic stress would increase the vulnerability of the species to interspecific competition and predation.

INSECTA

Collembola and Tipulidae were rarely recorded.

Odonata. Ischnura elegans was the only species of dragon fly whose nymphs were found in the canal and it was confined to the freshwater stations. This may correlate with the amount of weed available rather than with salinity itself as there are many established records of Odonata in both moderate and high salinities. Naids of Ischnura elegans were found in 73% sea-water (25°/ooS) after the floods of the Spurn peninsula in 1953 (Butler & Popham 1958) and Remane & Schlieper (1958) mention that the species has been found several times in the Baltic in salinities greater than 12°/ooS.

Hemiptera. Six species were collected in the canal. Although many records of the species have been taken from saline waters (table 21), Thorpe (1927) considered the Corixidae to be one of the groups most sensitive to sudden changes in salinity, and Macan (1963) only considers S.stagnalis and S.selecta (Fieb) to be mixohaline species.

In the saline flashes* near the canal Savage (1971) found permanent populations of S.dorsalis in salinities up to about 2°/ooS and S.stagnalis up to 15°/ooS. Callicorixa praeusta was taken occasionally but only in salinities less than 400 mgl^{-1}S.

*The term "flash" refers to water filled pits formed by subsidence of land above natural brine runs.

Table 21. Some records of Hemiptera from brackish waters.

Species	Total Salinity	Locality	Authority
Callicorixa praeusta	7°/oo	Spurn peninsula	BUTLER AND POPHAM 1958
	5-6°/oo	Baltic	LINDBERG 1948
Corixa affinis	8.5°/oo	Spurn peninsula	BUTLER AND POPHAM 1958
Sigara dorsalis	<3.5°/oo		MACAN 1962
	1-8-10°/oo	Seaton Sluice	SUTCLIFFE 1959
S.distincta	5-6°/oo	Baltic	LINDBERG 1948
S.fossarum	<3.5°/oo	Spurn peninsula	BUTLER & POPHAM 1958
	5.6°/oo	Baltic	LINDBERG 1948
S.stagnalis	28°/oo	Spurn peninsula	BUTLER & POPHAM 1958
	19°/oo	Baltic	LINDBERG 1948
	70°/oo	Baltic	REMANE & SCHLIEPER 1958
	21°/oo	Kent Coast	LANSBURY 1954

Trichoptera. Eleven species of caddis were identified in the canal though all were taken in the freshwater stretches (fig.15a, p92). British records indicate that with the exception of the mixohaline species, Limnephilus affinis, caddis are very sensitive to salinity. Butler and Popham (1958) found neither Trichoptera nor Ephemeroptera above 8°/ooS on the Spurn Peninsula and Sutcliffe (1961c) found that less than 50% of his specimens of Anabolia nervosa and L.stigma could survive at 7°/ooS for three days.

Records of caddis from the Continent suggest that there are a number of euryhaline caddis there. Silfvenius (1906) found 19 species between 5-6°/ooS in the Gulf of Finland, Siefert (1938) recorded Phryganea grandis and three other species at higher salinities in the Danish Wick, while Lindberg (1948) found nine species including Limnephilus flavicornis in the Baltic. Haage (1968) demonstrated in the laboratory that Phryganea grandis from freshwater could resist 5°/ooS though they showed a preference for freshwater over even dilute brackish waters.

As some of the species found in the canal are thus known to tolerate higher levels of salinity in other habitats, it seems possible that the dearth of macrophytes, emergent vegetation and even filamentous algae may make the saline stretches unattractive for oviposition and leave the larvae exposed to ecological pressures.

Coleoptera. The Coleoptera of the canal showed a distinct preference for the freshwater stretches, with the possible exception of Helophorus brevipalpis which is renowned for its mobility (Arnold and Macan 1969) and one exceptional sample taken at station 8 which included 16 individuals of Haliplus sp. The distribution is again more stenohaline than would be expected from previous records (table 22).

Table 22. Records of some Coleoptera from saline habitats.

Species.	Total Salinity	Location	Authority
Haliplus immaculatus	5°/oo	Spurn peninsula	BUTLER & POPHAM (1958)
H.wehnckei	<3.5°/oo	Spurn peninsula	BUTLER & POPHAM (1958)
H.lineatocollis	1-8.10°/oo	Seaton Sluice	SUTCLIFFE (1959)
Hydroporus palustris	5°/oo	Spurn peninsula	BUTLER & POPHAM (1958)
Hydroporus sp.	17°/oo	Salt Creek	THORPE (1931)
	21-25°/oo	New England Creek	HOWES (1939)
Helophorus brevipalpis	21°/oo	Spurn peninsula	BUTLER & POPHAM (1958)
Helophorus sp.	6-63°/oo	Westphalian salt pools	SCHMIDT (1913)

Lindberg's use of the term pseudohalobionts (1948) is probably relevant in this context. He used it to describe species whose way of life resembled that of halobionts in the northern and central parts of the Baltic but which in their southern distribution were typical limnobionts. His examples included five species of Haliplus and Noterus clavicornis. Remane and Schlieper (1958) suggest that the temperature - salinity relationship is one of the factors responsible for this.

Diptera.

Tanypodinae. Of the three species recorded in the canal, only Procladius sagittalis was found frequently enough to plot its distribution along a salinity gradient (fig.15a p 92) and it was apparent that the species was strongly halophye. The genus is known globally as a tolerant one and Thorpe (1931) reported prolific numbers of Procladius sp. in a low salinity pool fed by a warm spring water at Dos Palmas. Rawson and Moore (1944) found Procladius sp. throughout the saline lakes of Saskatchewan. In Australia Pollard (1971) recorded Procladius villosimanus Kieffer from 4‰ in L.Modewarre and Bayly &Williams (1966) took Procladius paludicola Skuse at 22°/ooS from Lake Kariah. In the Baltic,Remmert (1955a) took P.breviatus at salinities from 3-18°/ooS. Apparently no species of Procladius is recorded from British brackish-water sites.

Diamesinae. A single specimen of Prodiamesa olivacea was recorded at station 5.

Orthocladiinae. Cricotopus trifasciatus, Eukiefferiella brevicalcar and Metriocnemus atratulus were all taken on few occasions though the latter does appear to be halophye. Cricotopus sylvestris was taken frequently and showed a strong halophye tendency (fig.15b, p 93), with peak abundance at stations 8 and 9 [mean salinity 3,600-4,100 mgl^{-1}Cl (4,680 - 5,330 mgl^{-1}S)]. Stuart (1941) also recorded this species in the less brackish of the Millport shore pools, though it apparently could not survive 17°/ooS. Remmert (1955a,b) found it in the zone 0-10°/ooS.

Chironominae. Chironomus annularius, C.plumosus and C.cingulatus were generally distributed in the canal (table 17), the first two were recorded as far up the salinity gradient as station 10, and the latter up to station 8 with some indications that it may be halophye. C.riparius was quite distinctly halophye (fig.15b) with peak abundance between stations 9-13. C.riparius

(=thummi) was taken in salinities of 0-8°/ooS in the Baltic by Remmert (1955a,b) as were C.plumosus and C.annularius. Rawson and Moore (1944) found C.plumosus regularly up to salinities of 14.2°/ooS and though Lauer (1969) found it at 11°/ooS in Lenore Lake, it was absent from Soap Lake at 18°/ooS. In the laboratory he established a physiological limit of 16°/ooS and concluded that although the larva can perform both hypo-osmotic and hyperosmotic regulation, it could not sustain the former throughout its normal lifespan. Sutcliffe (1959) recorded C.riparius at salinities between 0-10°/ooS at Seaton sluice and found experimentally that it would not survive concentrations greater than 14°/ooS.

The only other chironomid that was recorded frequently in the canal was Glyptotendipes f.glaucus and must be considered to be strongly euryhaline, having been taken even at the most saline stations (fig.15c, p 94).

Cryptochironomus spp. Dicrotendipes sp. Pentapedilum sp. and Microtendipes sp. were infrequently recorded, and although Polypedilum spp. were not very abundant, they appear to be limnobiont and this is the conclusion reached by Thienemann(1954). Limnophyes sp.was irregularly recorded but appears to be euryhaline.

Ceratopogonidae and Tipulidae were normally not identified and were infrequently recorded, though they were present in the saline stretches.

Stratiomyidae were represented by Nemotelus notatusat three stations and Ephydridae by Ephydra riparia at stations 7-10 (fig.15c). Both thus appear to be halophye in the canal and are well known as approaching holeuryhalinity. (Howes 1939, Thorpe 1927, Thienemann1915).

b) Factors restricting the range of salinities in which a species is found.

It is apparent that many species recorded in the Trent and Mersey Canal are absent from salinities at which they have been recorded at other localities, viz. Bithynia tentaculata, Lymnaea spp., Asellus aquaticus, Ischnura elegans, Phryganea grandis and many of the Coleoptera. The discrepancies between the limits of the distribution in the canal and previously established tolerances are attributed to a number of factors.

1. Where physiological limits have been established in laboratory experiments they must be considered as extreme. Ecological tolerances are inevitably lower due to such factors as competition, predation and differential tolerances at different stages of the life-cycle (Beadle 1959). Kinne (1964) considered that for G.duebeni the narrowest range of tolerance is during early ontogeny.

2. Physiological races may exist, particularly in long established brackish waters which have a high stability. Natural selection would act strongly to equip a species for an environment where it was relatively free from competition. The brackish water and freshwater forms of Gammarus duebeni are considered to be distinct physiological races by some authors (Remane & Schlieper 1958) and Gasterosteus aculeatus L. has two subspecies gymnurus and trachurus which are both physiologically and morphologically distinct (Heuts 1947). Lagerspetz (1958) demonstrated that Asellus aquaticus from a brackish water population in the Baltic had a reduced tolerance for freshwater.

3. Phenotypic variation may permit gradual acclimation to salinities not normally tolerated. Beadle (1943a) thought that in acclimation experiments the extension of the range of tolerance was due to the development of a new mechanism for osmoregulation which was not originally functional.

4. Synergistic effect of other factors. Gresens (1928) showed that a decrease in temperature increased the survival time of Phryganea grandis and P.varia in high salinities and Sutcliffe

(1960b) demonstrated the effect of temperature on larval and pupal development of Limnephilus affinis at different salinities. Calcium may also be important and calcium ions are known to reduce the permeability of animal membranes, (Ellis 1937).

5. Freshwater preferences are displayed by many invertebrates which are euryhaline, e.g. Phryganea grandis (Haage 1969), Asellus aquaticus (Lagerspetz & Mattila 1961), Laccophilus maculosus Germ. (Hodgson 1951). The chemoreceptors of the latter have been investigated in detail (Hodgson et al 1955) and both Aedes aegypti (L). and Culex pipiens L. have also been shown to possess chemoreceptors for detecting salinity (Hudson 1956). Sutcliffe (1959) concluded from the movements of Coleoptera that they were generally absent from the salt marsh at Seaton Sluice not because they were vulnerable to influx of ions but because of a preference for more dilute waters.

6. Community relationships. Where a species is under physiological stress in a given salinity, it may survive if ecological conditions are favourable (eg. ample food supply, absence of competitors and predators), but not if ecological conditions are less than ideal. An ample food supply must be important where an organism has a high osmotic workload. It is probable in the Trent and Mersey Canal that the absence of Molluscs, Odonata and Trichoptera relates to the absence of plants for the provision of shelter, food, and sites for oviposition and pupation.

7. Geographical isolation. Salinity tolerant invertebrates may be absent from recently established brackish habitats merely because they have not yet been introduced. Although it is unlikely to be true for those insects having aerial stages, this is likely to be a limiting factor for the Molluscs. The Trent and Mersey Canal certainly experienced higher salinities historically (Holland 1976b) and these may have eliminated a fauna that is capable of tolerating present salinities. Furthermore the iron oxide pollution at Elworth may provide a barrier to downstream migration.

8. Saline instability can be harmful for invertebrates at the limit of their tolerance and Segerstråle (1957) attributes the extended range of lacustrine animals in the Baltic to the lack of tides. On a behavioural level, Haage (1969) found that larvae of Phryganea grandis would often be immobilised in a state of shock when crossing an interface between divergent salinities.

In the light of these various factors, it seems that the distributions of invertebrates in the canal in relation to salinity are meaningful in terms of ecological tolerance in variable conditions. The molluscs are a possible exception and may be restricted as a result of their immobility. The allocation of species to the various categories of table 18 was done with some confidence that they provide realistic tolerance of species for unstable saline conditions in Britain. The classification is, in the most part, in agreement with the one derived for the fauna of Crow Brook, both in terms of allocation of species to categories and in terms of salinity limits, though it was impossible to determine whether Asellus aquaticus and Potamopyrgus jenkinsi had a halophye disposition as this was masked by the discontinuity in the salinity gradient at this point.

c) Changes in the number of species, (fig.18)

Number of species plotted against stations in order of ascending salinity shows a decline. The first feature is a significant decline from stations 1 and 2 to stations 3, 4 and 5, even though the last three were not detectably saline. This indicates that these stations may have been saline historically and that recovery of the fauna has been slow. The presence of Gammarus duebeni and prolific numbers of Potamopyrgus jenkinsi along this stretch indicate that the water may be of a richer ionic composition. The next drop in species numbers occurs between the freshwater stretch and the saline stretch as would be expected, though rather more species were taken from stations 8-11 than anticipated. Rice and Kelting (1955) demonstrated what is in fact an intuitive phenomenon, that number of species

collected from an area increases with number of samples taken, and it seems that this is the most likely explanation for the apparent increase in species number between stations 11 and 8 (table 10, p 75). Number of species at station 6 is particularly low but this may be due to other causes which have already been discussed. The halobic index as derived from the synecology of Crow Brook, (table 8, p 66) rates stations 14 and 15 less extreme, and stations 6, 7 and 12 more extreme environments than the rest of the saline stations (table 23). Station 12 was only sampled five times and the species diversity was probably underestimated, resulting in a low halobic index.

Table 23. Halobic index for each station on the canal system.

Station No.	1	2	3	4	5	15	14	13	12	11	10	9	8	7	6
Halobic index.	19	19	18	18	18	10	10	6	5	6	6	6	6	5	3

The halobic index therefore appears to be a valid tool for biological assay, being sensitive to a paucity of fauna which was undetectable in terms of numbers of species alone. The low ratings of stations 6 and 12 indicate that certain conditions must apply for meaningful use of the index;

i) that there should be an adequate sampling intensity,
ii) that the presence of other pollutants may produce a lower rating than is merited by salinity alone.

d) Changes in the relationship between number of species and numbers of individuals.

The general principle that as environmental conditions become more extreme, species diversity declines and number of individuals increases, is not immediately apparent for the fauna of the Trent and Mersey Canal. Variations in sampling intensity were considerable from station to station and the fauna itself was very variable.

For example 50% of all individuals at station 2 were Asellus aquaticus, and 487 Gammarus tigrinus were taken in just one sample at station 14 and thus account for rather low indices of diversity at these stations. Computation of the index (table 24) otherwise greatly overcomes the problem of variations in sampling intensity from station to station.

Table 24. Index of diversity (∝), (excluding Oligochaetae and Potamopyrgus jenkinisi).

	Low Salinity				Station number							High salinity			
	1	2	3	4	5	15	14	13	12	11	10	9	8	7	6
No. samples taken.	11	13	5	4	13	2	7	6	5	11	10	13	13	8	12
No. Species.	39	34	20	25	24	10	13	13	9	13	19	18	16	8	5
No. individuals.	482	1660	192	203	420	76	1211	349	77	691	355	1341	675	526	22
Index of diversity (∝)	8.7	5.2	5.6	6.8	5.2	2.6	1.8	2.7	2.2	2.3	4.0	2.2	2.6	1.3	2.0.

It is evident that although the halobic index discerns changes in the biocoenosis with increasing salinity, there is no gradual decline of species diversity from low salinity as was seen in Crow Brook. This is attributed to the downstream position of the less saline stretch of the Trent and Mersey Canal. Waves of high salinity water passing through this zone may pauperize the fauna and in this situation, drift and migration from freshwater

zones cannot aid recovery, as an upstream barrier of high salinity, and possibly other pollutants, must be traversed.

SUMMARY

1. The ecology of the Trent and Mersey Canal was studied over the period of one year in relation to salinities ranging from freshwater to a mean salinity of nearly 5,000 mgl^{-1} Cl (8,400 mgl^{-1} S) along the length of the canal.

2. Tolerances of the major taxa for salinity are discussed, and summarized in table 18, p 97). These were generally in keeping with tolerances established at other mixohaline locations, though the reasons for apparently reduced tolerance of some species in the canal are discussed.

3. In particular, the co-existence of three Gammarus spp. is discussed in relation to their zonation with salinity and the complementation of their seasonal cycles.

4. The halobic index constructed from the data in Chapter III was applied to particular stations along the canal and appears to be a sensitive tool, although cautions are invoked for its application.

5. The index of diversity was computed for all stations and was considerably lower for the saline stretches than the freshwater stretches. This was taken to indicate proliferation of numbers in a few salt tolerant species.

CHAPTER V

THE FAUNAS OF SOME NATURAL BRINE SPRINGS AND BRINE PITS.

INTRODUCTION.

Historically there are many records of natural brine springs arising where the rock-head is almost exposed and runs of natural brine find their way to the surface (Calvert 1915, Sherlock 1921). However, the number of these natural springs appears to have declined considerably in recent years, partly as a result of erosion of the rock head, but chiefly since the advent of brine pumping which has lowered the natural brine table. The locations of over a dozen brine springs catalogued by Sherlock (op.cit.) were explored in the present study, but only three of these were found to be extant, and one further site was located in Staffordshire.

The first was on the banks of the R.Weaver just south of Nantwich where brine flowed at the surface in the nineteenth century, and was first recorded by Calvert (p734), "the spring.....overflowed gently into the Brook, the surface water containing 11b 10oz of salt per gallon." ($164^{o}/oo$ NaCl). Sherlock (op.cit) reports that the brine rose naturally from a depth of 42m. The spring was still in existence at the beginning of this study in 1971, though the brine was considerably weaker. Unfortunately alterations to the course of the River Weaver in 1973 obliterated the site.

Sherlock also mentioned a salt spring near Aldersey Hall, Clutton and this was investigated by Burke (1942a) who established the first non-coastal record of the marine alga Percursaria percursa Rosenv. there. The level of the spring and the amount of brine has greatly declined in recent years and, whereas the squire once swam regularly in the "spore", there is now little more than 0.3m of water above the ooze.

The third location was a brine pit of some antiquity at Lower Wych, Malpas, which was worked until 1856. According to Ormerod (1848, p269), the pit was man-made, and a shaft had been sunk 137m through clay to tap the brine run which entered the shaft at a depth of 18m. Burke (1942b) visited the pit to make observations on the Diptera, and the level and concentration of the brine is still as he described it.

One other historic brine working site was investigated, but this time in Staffordshire where Sherlock (p70) described historic brine springs at Shirleywich and Weston. A salt marsh flora (Dr.K.Goodway, pers.comm) indicated that the rock head approached the surface at Pasturefields near Weston, and although the brine wells at nearby Shirleywich were dry, the drainage channel of the salt marsh proved worth investigation.

Burke's study of the Lower Wych Pit (1942b) is the only biological investigation that has been made of a natural brine spring in Britain and although Bayly (1972) reviews an extensive international literature from inland saline waters, only a few sites where salinity is chloride based have been studied. Schmidt (1913) and Thienemann (1913, 1915) surveyed the invertebrate fauna of the Westphalian salines and those at Bad Oldesloe (1925) where salt deposits have also been exploited, and Baas-Becking (1928) enumerated brine organisms not only from Westphalia, but also from the salterns of San Francisco. Schuster and Mohrig (1970) catalogued twenty-eight species of mosquito from the salt-processing pools of Magdeburg, and Caspers (1952) listed a total of twenty-three species from the Bulgarian salt pans. In North America the faunas of the saline lakes of the Great Basin have been described by several authors (Schwarz 1891, Vorhies 1917, Alleé 1926, Patrick 1936, Woodbury 1948, Zahl 1967). Hedgpeth (1956), Hesse et al (1937) and Beadle (1943b) discuss other hypersaline waters in the Northern hemisphere.

Description of sites.

1. Shrewbridge brine pool (grid reference SJ649513

Brine from the spring seeped through a culvert under a road bridge into a roughly rectangular pool 25m long and 8m wide (plate 3), and although there was an outlet into the R.Weaver, flow was negligible. At the edges the pool was 0.5m, deepening to a maximum depth of about 1m centrally. The surrounding vegetation was dominated by Juncus spp.

2. Aldersey Spore (grid reference SJ 457565).

The brine spring (plate 4) is circular and about 5m in diameter with about 0.3m of brine over thick mud. A deep carpet of Percursaria percursa covers the surface of the Spore in summer. Brine seeps via a field drain to a shallow ditch 1m wide (plate 5) which joins an extensive field drainage system. Levels of brine in the ditch are rarely more than 0.3m.

3. Pasturefields salt-marsh (grid reference SJ 989 251 to SJ 993 246).

The salt-marsh is situated alongside the R.Trent at Weston and is drained by a 2m wide channel, with a depth of 0.3m, for a distance of over 500m (plate 6). The surrounding vegetation is mainly Juncus spp. though the ditch contains extensive beds of Apium graveolens L., Lemna minor, Callitriche sp. and Cladophora sp.

4. Lower Wych brine pit (grid reference SJ 488 441).

The pit (plate 7) is conical with diameter of 8m and a fine mud bottom sloping steeply towards the central well. Burke (1942b) took samples from as far down a 9m, though the well is reputedly nearly 140m deep (Sherlock, 1921). The pool is frequently visited by cattle that are attracted to the salt which crystalises at its edges.

Plate 3. Shrewbridge brine pool.

Plate 4. Natural brine spring, Aldersey.

Plate 5. Aldersey ditch, sampling station No.1.

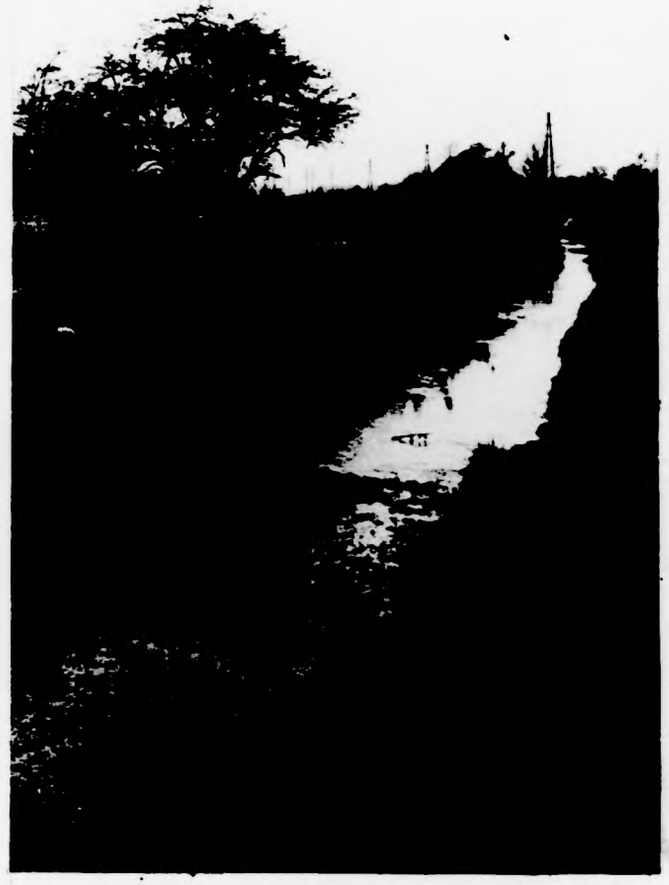


Plate 6. Drainage ditch, Pasturefields salt marsh.

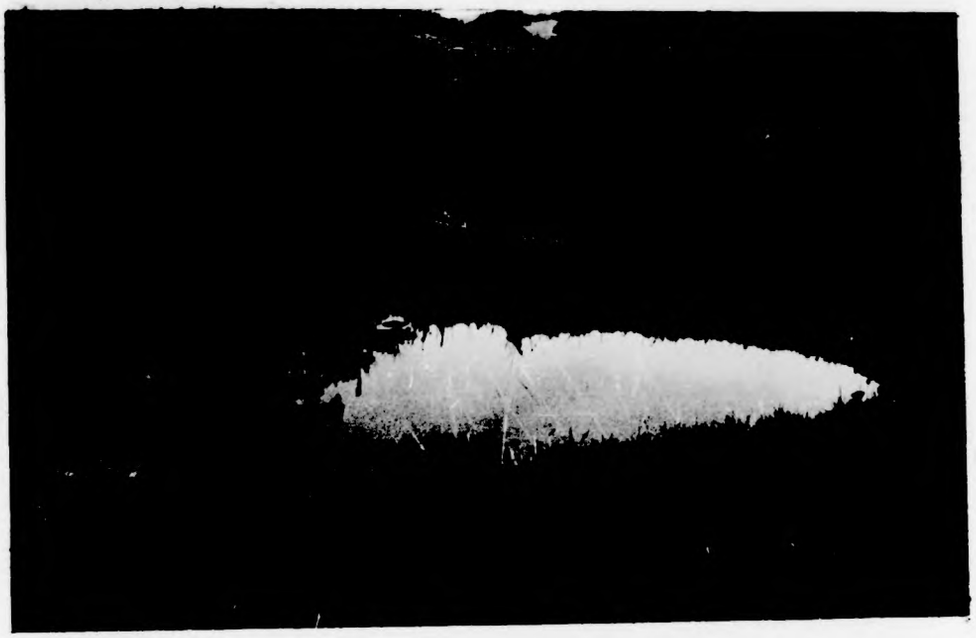


Plate 7. Lower Wych brine pit.

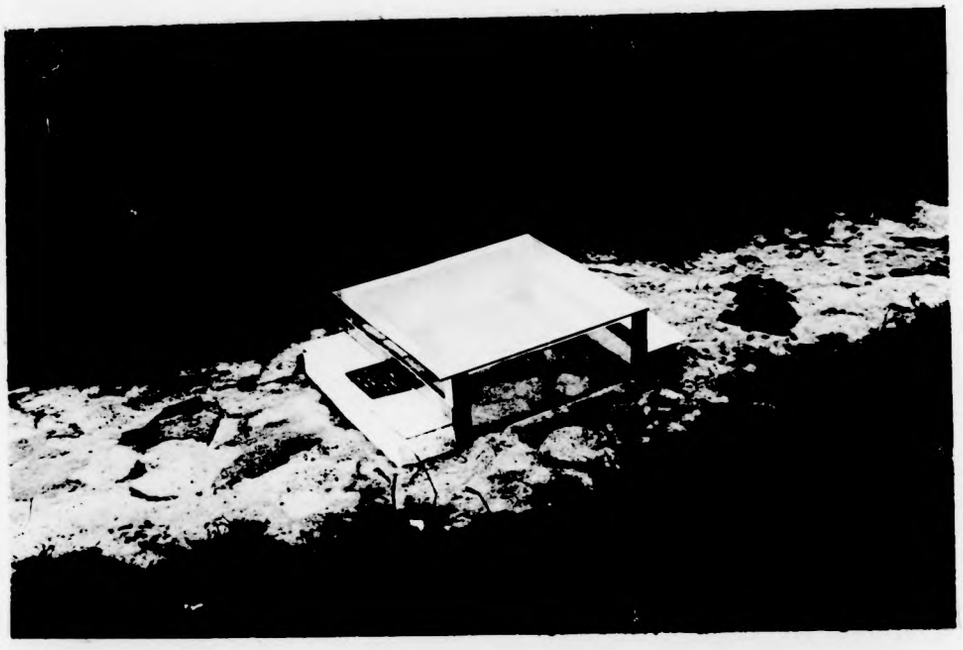


Plate 8. Emergence trap for aquatic insects.

METHODS.

Water samples were collected for determination of chlorides, conductivity and pH from each of the sites though at Aldersey samples were also taken from the ditch immediately adjacent to the spring (station 1) and also from the drainage system 200m away (station 2). As the Shrewbridge pool was somewhat larger than the other brine pools an estimate was made of the variation in salinity from point to point on 18th September 1973 when 50 samples were taken from around the pool and their conductivities determined. A sample was titrated for hardness on the same occasion. At Pasturefields samples were taken at four equidistant points along the ditch, however, as variation was low, data for the four stations were pooled.

Samples of the fauna were collected in sweeps of a Freshwater Biological Association pond net and transferred to 4 litre buckets for transportation to the laboratory. As the faunas of Shrewbridge pool, Aldersey Spore and the Lower Wych brine pit were dominated by Diptera, larval determinations frequently proved difficult, and during the summer of 1973 emergent insects were trapped using Green's (1970) modification of the Solbé trap (Plate 8). Traps were not used at Pasturefields or at station 2 at Aldersey. The glass plates were thinly coated with the adhesive 'Bandite Tanglefoot' using a photographic print roller. The plates presented a surface area of $0.1m^2$ to which emergent insects would adhere. The plates were transported to the field in sealed boxes and left on the trap for a 24h period. They were then taken to the laboratory where insects were removed using a 30% acetone : 70% alcohol solvent. I am indebted to Garth Foster (West of Scotland Agricultural College) for assistance in identifying the Coleoptera and to Brian Cogan, Peter Cranston and K.G.V.Smith of the British Museum (Natural History) who assisted with the identification of the Ephydridae, Chironomidae and Dolichopodidae respectively.

RESULTS

Water quality.

Salinity and pH data for each locality are presented in table 25. The pH was almost neutral at all localities and had a standard deviation of less than 0.5 pH units. At Shrewbridge exceptional salinities were occasioned in January 1972 by blasting during repair of the road bridge, though these had dropped to fairly stable levels by April of the same year. Salinity during the period of investigation is plotted for this site in figure 20.

The correlation between salinity (x) and conductivity (y) was computed for each locality and the regression lines are presented in figure 21. As the extreme salinities of the Lower Wych pit necessitated a dilution of samples before titration (x50), a high experimental error would be expected and probably accounts for a rather low correlation coefficient ($r = 0.765$). The regression of conductivity on chlorinity at this site is described by $y = 0.26x - 4.14$ and is not plotted in figure 21 due to the extreme values.

The fifty samples taken at Shrewbridge on 18.9.73 to establish variability across the pool gave a mean conductivity of 1.74×10^4 μmhos cm^{-1} with a standard deviation of 0.31×10^3 μmhos cm^{-1}. These were equivalent to a mean salinity of 7,070 mgl^{-1} Cl and standard deviation of 95 mgl^{-1} Cl, which means that sampling error from point to point around the pool would be negligible. Concentrations of calcium and magnesium at this time were 168 mgl^{-1} and 62 mgl^{-1} respectively, giving a total hardness of 230 mgl^{-1}.

Biological components.

Algae. *Percursaria percursa* was abundant in Aldersey Spore and an unidentified alga of similar form was taken from Lower Wych Pit. *Enteromorpha intestinalis* was found at both Shrewbridge and Pasturefields.

Bacteria. Iron bacteria were observed at the inlet to the Shrewbridge pool, at station 1 on the Aldersey ditch and in Pasturefields ditch. Purple sulphur bacteria were abundant in Aldersey Spore and were also observed at the Lower Wych pit on 14th February 1972.

Invertebrates. Species lists for each locality are presented in tables 26-30, where numbers of individuals taken by pond net are listed, and although these do not relate to given areas, they do give an indication of relative abundance. Numbers of individuals emerging per unit area are also tabulated for each occasion on which the emergence traps were set, and the halobic index was computed for each site according to table 8, (p66).

DISCUSSION

1. Flora and photosynthetic bacteria.

The presence of freshwater macrophytes at Pasturefields indicates that Apium graveolens, Callitriche sp. and Lemna minor are all tolerant of low salinities at least. A.graveolens was found throughout the ditch and was growing in salinities of at least 4,050 $mgl^{-1}Cl$. L.minor and Callitriche sp. were localised but were recorded in salinities of at least 1,180 $mgl^{-1}Cl$.

Although strands of Enteromorpha intestinalis were present at Shrewbridge and Pasturefields, the thick carpet of Percursaria percursa was the most striking feature of the brackish water flora. In summer the Spore was completely covered by a solid mat of the alga, which closely resembled the Vaucheria carpets in the Sperenberg area of the Baltic. The description of Kolbe is quoted by Remane and Schlieper (1958).... "these cover the ground with a coherent carpet impenetrable to gas bubbles. The exchange of gases between the bottom ooze and the open water being impeded, this results in a disturbance in the normal process of decay in the former. Apart from the usual formation CH_4 - there is another visible consequence, a vigorous production of H_2S". At

 Text continues on P.147.

Table 25. Salinity and pH of the Cheshire and Staffordshire brine springs.

Locality	Salinity ($mgl^{-1}Cl$) Mean	± standard deviation	Range	pH
Lower Wych Pit	57,400	52,500-62,300	53,100-69,500	7.6
Aldersey Spore	9,580	7,430-11,720	4,080-12,580	7.4
Aldersey Ditch (station 1)	6,670	5,050- 8,300	3,100- 9,200	6.7
Aldersey Ditch (station 2)	3,120	1,580- 4,660	1,580- 5,460	7.3
Shrewbridge	4,570**	2,520- 6,610+	2,350-33,800*	7.3
Pasturefields	806	260- 1,355	26- 1,740	7.1

**since 10.1.72 when exceptional salinities were experienced.

* a catastrophic value, see figure 20.

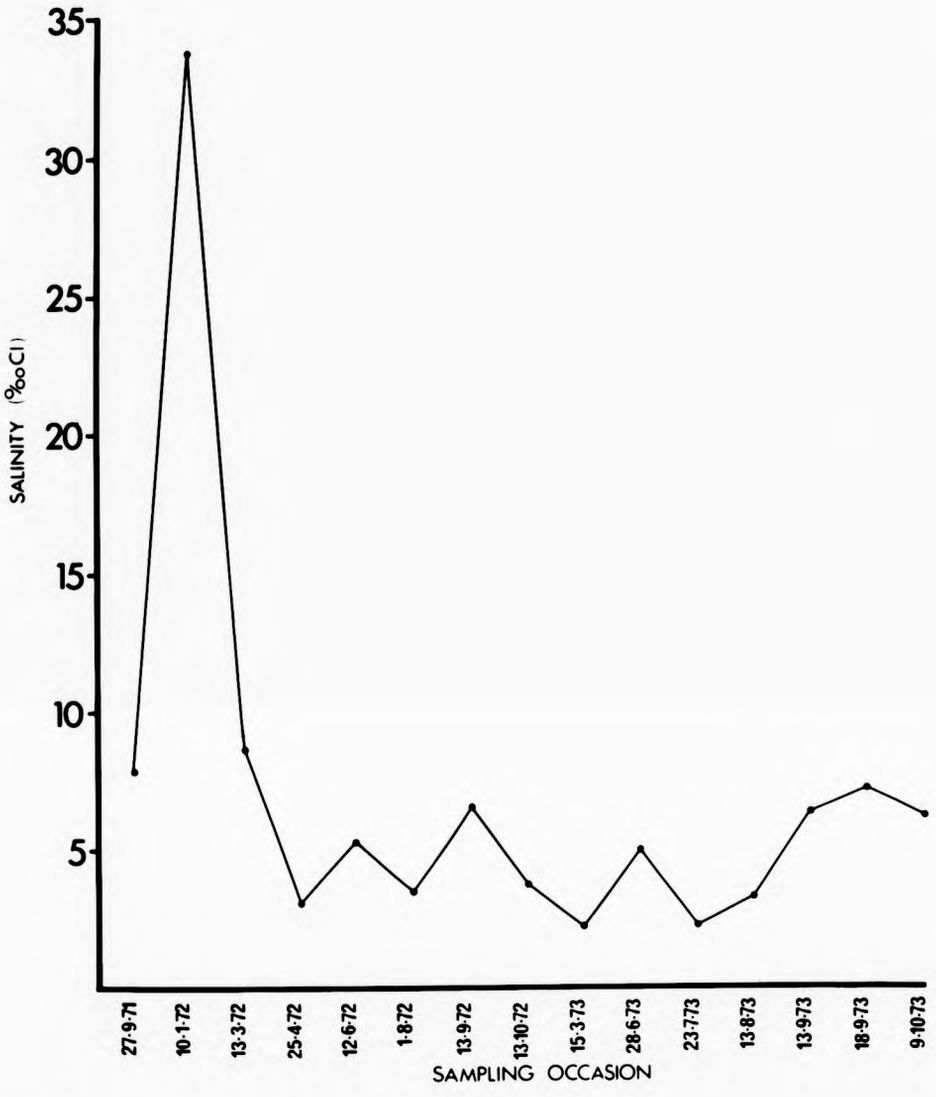


FIG. 20. VARIATION OF SALINITY AT SHREWBRIDGE BRINE POOL

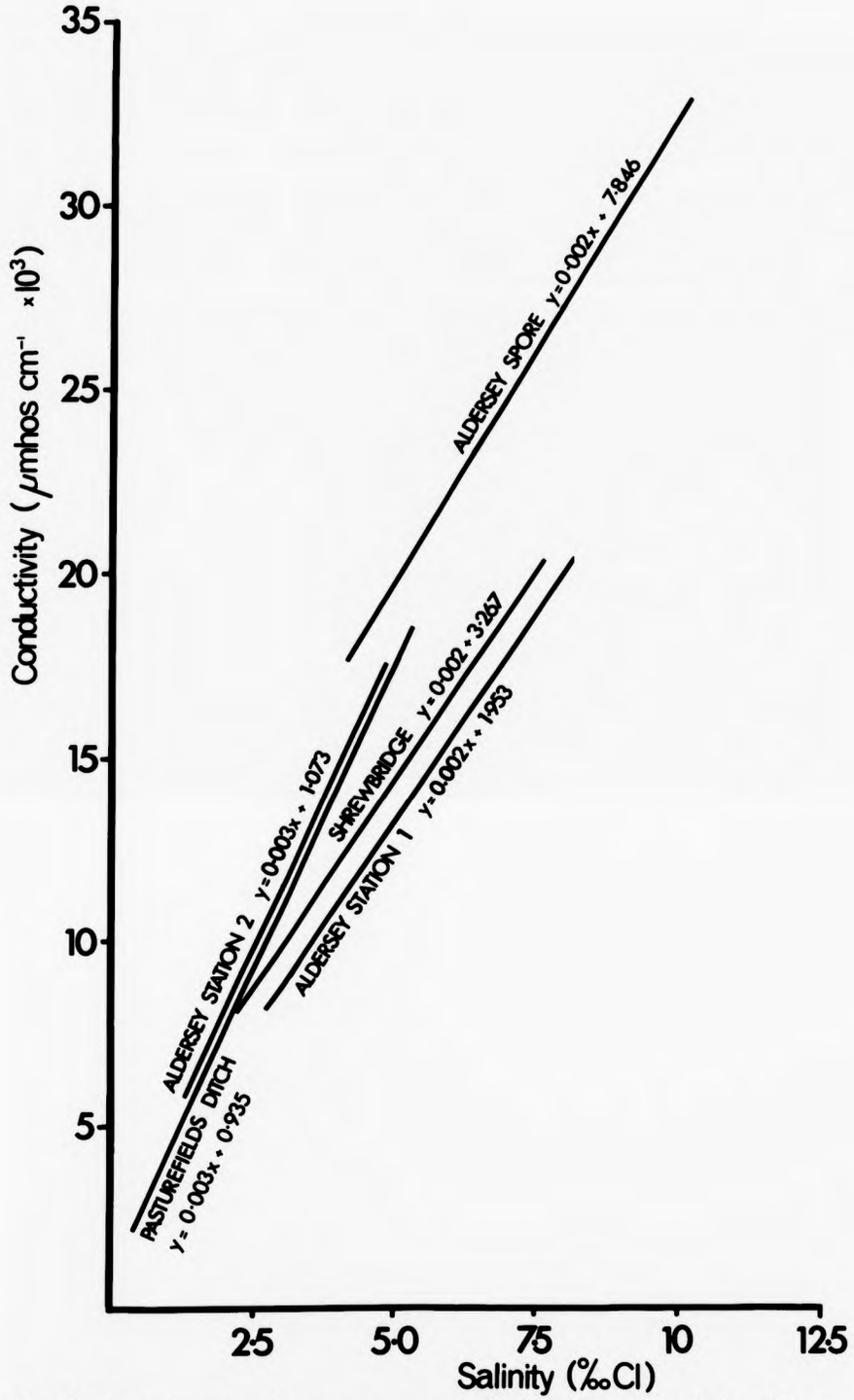


Fig. 21. Relationship between conductivity (y) and salinity (x) in the brine pits and brine springs.

Table 26. Species and numbers of individuals found at Lower Wych Pit.

	No. indivs. in pond net samples				No. emergent insects in 0.2m^{-2}					
	3.11.71	14.2.71	12.6.72	1.8.72	25.5.73	26.6.73	23.7.73	13.8.73	13.9.73	9.10.73
INSECTA										
Thysanoptera										
Thripidae										
unidentified								2		
Hemiptera										
Corixidae										
Sigara Halicorixa sp. ♀										2
Coleoptera										
Hydrophilidae										
Haliplus lineatocollis (Marsham)				3						
Hymenoptera										
unidentified							5			
Diptera										
Stratiomyidae										
Nemotelus notatus Zetterstedt			1	2						
Sphaeroceridae										
Leptocera sp.									1	2
Ephydridae										
Atissa pygmaea Haliday					1	1				2
Ephydra riparia Fallen	numerous						1		4	2

Table 27. Species and numbers of individuals found at Shrewbridge Brine Spring.

	No. indivs. in pond net samples	No. emergent insects in $0.3m^{-2}$						No. emergents as % total
		10.4.73	25.5.73	28.6.73	23.7.73	18.9.73	9.10.73	
Annelida								
Oligochaeta								
Enchytraeidae								
Lumbricillus reynoldsoni Backlund	x							
Naidae								
Nais elinguis Müller	x							
Crustacea								
Isopoda								
Asellus aquaticus Linnaeus	1							
Arachnida								
Hydracarina (unidentified)							2	1.5
Insecta								
Collembola (unidentified)	1					1		0.7
Odonata								
Coenagridae						1		0.7
Ischnura elegans (van der Linden)	13							
Thysanoptera								
Thripidae (unidentified)								
Hemiptera								
Gerridae								
Gerris thoracicus Schummel.	1							
Corixidae								
Sigara stagnalis (Leach)	163*					1		0.7
Homoptera (unidentified)						3		2.2
Trichoptera								
Limnephilidae								
Limnephilus affinis Curtis	1					1		0.7
Coleoptera								
Dytiscidae								
Agabus bipustulatus (Linnaeus)	2							
Colymbetes fuscus (Linnaeus)	1							
Dytiscus semisulcatus Mueller	1							
Hydrophilidae								
Helophorus grandis (Linnaeus)	2							
H.brevipalpis Bedel	3							
H.minutus Fabricius	2							
Enochrus 4 punctatus (Bedel)	2							
unidentified Coleoptera	5							
Hymenoptera (unidentified)					2	1		2.2
Diptera								
Tipulidae (unidentified)						1		0.7
Psychodidae								
Telmatoscopus soleatus (Walker)			1		1			1.5
Psychoda phalaenoides (Linnaeus)					2			1.5
P.severini s.parthenogenetica Tonnoir					4			3.0
P.grisescens Tonnoir						1		0.7
Trichomyia urbica Haliday					1			0.7
Culicidae								
Culex pipiens Linnaeus	2				1	1		1.5
Aedes detritus Haliday	1							0.7
Ceratopogonidae (unidentified)					1		3	3.0

Table 27 continued.

	No. indivs. in pond net samples	No. emergent insects in 0.3m^{-2}							No. emergents as % total
		15.3.73	10.4.73	25.5.73	28.6.73	23.7.73	18.9.73	9.10.73	
Insecta									
Diptera									
Chironomidae									
Procladius sagittalis (Kieffer)	164		4				1		3.7
Cricotopus sylvestris var.fuscimanus (Meigen)	2	2						5	5.2
C.sylvestris var.ornatus (Meigen)	23					23	6	2	23.0
Halocladius varians (Staeger)	4								
Orthocladius obtexens Brundin	2								
unidentified Orthocladiinae	6								
Chironomus riparius Meigen C.aprilinus Meigen C.salinarius Kieffer	291					2		2	3.0
Einfeldia sp.	1								
Limnochironomus nervosus (Staeger)	1								
Pentapedilum sp.	1								
Cecidomyidae (unidentified)		1				1			1.5
Empididae									
Hilara obscura Meigen						2			1.5
Dolichopodidae									
Dolichopus griseipennis Stannuis						1			0.7
D.trivialis Haliday						1			0.7
Campsicnemus curvipes (Fallèn)						3			2.2
Teuchophorus monacanthus Loew						2			1.5
unidentified Dolichopodidae (♂♂)						6			4.4
Ephydridae									
Notiphila cinerea Fallèn					1				0.7
Atissa pygmaea (Haliday)						1			0.7
Ephydra riparia Fallèn	n			1	1	9	6	5	16.3
Scatella stagnalis (Fallèn)							15		11.1
unidentified Ephydridae							1	1	1.5

* nymphs present
n numerous

Table 28. Species and numbers of individuals found at Aldersey Spore and Aldersey station 1.

	Aldersey Spore										Aldersey station 1.									
	Total no. indivs. in pond net samples*	No. emergent insects in $0.3m^{-2}$								No. indivs. as % total emergents	Total no. indivs. in pond net samples*	No. emergent insects in $0.2m^{-2}$								No. indivs. as % total emergents
		15.3.73	10.4.73	25.5.73	28.6.73	23.7.73	13.8.73	18.9.73	9.10.73			15.3.73	10.4.73	25.5.73	28.6.73	23.7.73	13.8.73	18.9.73	9.10.73	
Annelida																				
Oligochaetae																				
Lumbricillus reynoldsoni Backlund.	13										x									
Crustacea																				
Isopoda																				
Asellus aquaticus L.											2									
Arachnida																				
Hydrocarina (unidentified)							9			1.3						2		76	3	30
Insecta																				
Collembola (unidentified)											4						3			1.1
Thysanoptera (unidentified)																				
Hemiptera																				
Homoptera (unidentified)					1			3								1		2	1	1.5
Trichoptera																				
(Limnephilidae)																				
Limnephilus affinis Curtis										0.6	67									
Coleoptera																				
Dytiscidae																				
Agabus bipustulatus (Linnaeus)	5										3									
Hydroporus memnonius Nicolai	3										2									
H. incognitus Sharp	2										1									
H. tessellatus Drapiez	4										1									
H. planus (Fabricius)											1									
Haliplus lineatocollis (Marsham)	1									0.14										
Hydrophilidae																				
Ochthebius dilatatus Stephens	6										1									
O.minimus (Fabricius)	7										2					1				0.4
Anacaena globulus (Paykull)	11																			
Helophorus grandis (Linnaeus)	9				1					0.14	51					4				1.5
H. brevipalpis Bedel	8				9	3				1.7										
H. minutus Fabricius	10										7									
unidentified larvae											20									

Table 28 continued.

	Aldersey Spore										Aldersey station 1.									
	Total no. indivs. in pond net samples*	No. emergent insects in $0.3m^{-2}$								No. indivs. as % total emergents	Total no. indivs. in pond net samples*	No. emergent insects in $0.2m^{-2}$								No. indivs as % total emergents
		15.3.73	10.4.73	25.5.73	28.6.73	23.7.73	13.8.73	18.9.73	9.10.73			15.3.73	10.4.73	25.5.73	28.6.73	23.7.73	13.8.73	18.9.73	9.10.73	
Insecta																				
Hymenoptera (unidentified)				3	4		9	9		3.5						3	2		2	2.6
Diptera																				
Tipulidae (unidentified.)	1									0.14	6									
Psychodidae																				
Pericoma avicularia Tonnoir																1				0.4
Telmatoscopus ?ambiguus (Eaton)																	1			0.4
T. soleatus (Walker)				3		1	3	3		1.4						2	4	8		5.2
Clytocerus ocellaris (Meigen)													1							0.4
unidentified pupae	16										4									
Culicidae																				
Aedes detritus Haliday											12									
Chaoboridae																				
Chaoborus sp.	2																			
Ceratopogonidae (unidentified)	40+			48	71	186	270	21		84.3	numerous	11		5	2	2	1	5		9.6
Chironomidae																				
Procladius sagittalis (Kieffer)	4		3		4					1.1	522			3			1			1.5
Cricotopus sylvestris (Fabricius)											2		1				3	1		1.9
other Orthocladinae																		1		0.4
Chironomus ?riparius Meigen	2			12		2				2.0	5000+						9	18	3	11.1
?Einfeldia sp.	1										2									
Polypedilum nubeculosum (Meigen)	2																			
Cecidomyidae (unidentified)																	4			1.5
Tabanidae (unidentified)	1									0.14										
Dolichopodidae																				
Campsicnemus curvipes (Fallen)												1								0.4
Sympycnus desoutteri Parent										0.14										
Sepsidae																				0.4
Themira sp.										0.4										
Syrphidae																				
group III larvae	8										2									

Table 28. continued

	Aldersey Spore										Aldersey station 1.									
	Total no. indivs. in pond net samples*	No. emergent insects in $0.3m^{-2}$								No. indivs. as % total emergents	Total no. indivs. in pond net samples*	No. emergent insects in $0.2m^{-2}$								No. indivs. as % total emergents
		15.3.73	10.4.73	25.5.73	28.6.73	23.7.73	13.8.73	18.9.73	9.10.73			15.3.73	10.4.73	25.5.73	28.6.73	23.7.73	13.8.73	18.9.73	9.10.73	
Insecta																				
Diptera																				
Sphaeroceridae		3								0.4										
Copromyza sp.																				
Ephydridae																				
Notiphila cinerea Fallèn	1						3			0.4	2									
Ephydra riparia Meigen	420	6								1.1	16					1	1	1		1.1
Hydrellia ?modesta Loew.	1																1			0.4
H.griseola (Fallèn)																				
Scatella stagnalis (Fallèn)	5		3	3						0.8	26							69	2	26.3
S. ?tenuicosta Collin								3		0.4										
Chloropidae (unidentified)	2																			
Scatophagidae																				
Scatophaga stercorcaria (Linnaeus)												1					1			0.7
unidentified immature Diptera											5									
		Total no. emergents=707 Halobic index=5										Total no.emergents=270 Halobic index=6								

*Eight samples were taken between 3.11.71 and 13.10.72

Table 29. Species and numbers of individuals found at Aldersey station 2.

	No. individuals in pond net samples							Relative abundance of each species in samples.
	10.1.72	14.2.72	13.3.72	25.4.72	12.6.72	1.8.72	13.10.72	%
Annelida								
Oligochaeta (unidentified)		2						0.1
Mollusca								
Gastropoda								
Potamopyrgus jenkinsi (Smith)	1	11	20	307	174	362	173	54.1
Planorbis sp.			1					0.05
Crustacea								
Amphipoda								
Gammarus pulex (Linnaeus)		1		2				0.15
Isopoda								
Asellus aquaticus Linnaeus		10	19	397	16	121	68	32.6
Insecta								
Trichoptera								
Limnephilus affinis Curtis		4						0.2
Coleoptera								
Haliplus palustris Marsh		1		1				0.1
H.lineatocollis (Marsham)		1			1	7		0.5
Helophorus brevipalpis Bedel							1	0.05
Hydroporus palustris (Linnaeus)		1		1				0.1
Agabus bipustulatus (Linnaeus)	1							0.05
Anacaena globulus (Paykull)						2		0.1
unidentified		1			5			0.3
Diptera								
Culex pipiens Linnaeus							2	0.1
Procladius sagittalis (Kieffer)				9	20	17		2.4
Chironomus riparius Meigen		13	5	61	12	4	77	8.9
unidentified Chironomidae							1	0.05
Ephydra riparia Meigen			1					0.05
TOTAL	2	45	47	777	230	513	322	

Table 30. Species and numbers of individuals recorded from the drainage ditch at Pasturefields Salt Marsh.

		No.individuals in pond net samples			No.indivs. as % total
		24.1.72	21.2.72	20.3.72	
Platyhelminthes					
Tricladida	Polycelis tenuis Ijima			2	0.1
Annelida					
Oligochaeta	unidentified	x	x		
Mollusca					
Gastropoda	Potamopyrgus jenkinsi (Smith)			1	0.06
	Lymnaea pereger (Müller)	89	118	72	19.2
Crustacea					
Amphipoda	Gammarus pulex (Linnaeus)	3			0.2
Isopoda	Asellus aquaticus Linnaeus	5	25	253	19.4
Insecta					
Collembola	unidentified		3		0.2
Hemiptera	Notonecta glauca Linnaeus			1	0.06
	Sigara dorsalis (Leach)			1	0.06
Neuroptera	Sialis lutaria (Linnaeus)	1		32	2.3
Ephemeroptera	Centroptilum pennulatum Eaton	4	2	1	0.5
Trichoptera	Limnephilus sp.	2	1		0.2
Coleoptera	Haliplus lineatocollis (Marsham)		1	2	0.2
	H. wehnckei Gerhardt			3	0.2
	Hydroporous palustris (Linnaeus)			3	0.2
	Agabus sturmii (Gyllenhal)		2		0.1
	unidentified			1	0.06
Diptera	Pentaneura sp.			6	0.4
	Macropelopia sp.		1	1	0.1
	Procladius sagittalis (Kieffer)	180	29	140	24.0
	Cricotopus sylvestris (Fabricius)	1	1	3	0.3
	Chironomus riparius Meigen	5			0.3
	C.annularius (Degeer)	82	35		8.0
	C. dorsalis Meigen	56	9	2	4.6
	C. lugubris Zetterstedt	130	65	42	16.3
	Endochironomus sp.		1	1	0.1
	Einfeldia sp.	1	2	6	6.2
	Polypedilum nubeculosum (Meigen)		8	12	1.4
	Pentapedilum sp.	3			0.2
	Microtendipes sp.			1	0.06
	unidentified Chironomidae			3	0.2
	unidentified dipterous larvae			1	0.06
TOTAL		562	303	589	

Aldersey the same process was manifested by the formation of gas bubbles both in and below the algal carpet and by the strong smell of H_2S if the carpet were disturbed. The underside of the carpet consisted of black decaying algal filaments coated with white deposits of sulphur and clumps of purple sulphur bacteria, chiefly Lamprocystis roseo-persicina. Baas-Becking (1925) found the sulphur bacteria remarkably indifferent towards salinity and reports that there have been numerous records of purple photosynthetic bacteria from sea water, and he himself found them in Redwood City brine. The bacteria do show a strong preference for well buffered alkaline water and require a source of hydrogen sulphide which may be derived from organic remains or by the reduction of sulphate by bacteria. Sulphate levels are generally high in brine and sodium sulphate constitutes 0.5% of the Holford brine (25% sodium chloride) and Sherlock (1921) found that some of the Cheshire brines, of only 3% sodium chloride, contain up to $5^{o}/oo$ magnesium sulphate and $5.3^{o}/oo$ calcium sulphate. These would probably be precipitated, as carbonate levels are high, and are probably well represented in the bottom ooze of the brine springs. Furthermore, the decaying filaments of P.percursa at Aldersey are probably a sufficient source of hydrogen sulphide for the endothiobacteria. Lamprocystis roseo-persicina was also present in the Lower Wych pit, coating the decaying pupal cases of Ephydra riparia. Gaseous exchange with the surface is unimpeded at this site and hydrogen sulphide is unlikely to be the source of hydrosulphide ions. Sulphate in the concentrated brine probably supports sulphate reducing bacteria which in turn supply the purple photosynthetic bacteria with hydrosulphide ions.

Iron bacteria were also found at the saline sites and they too are presumably indifferent to salinity. The marshy ground at the source of the Shrewbridge spring was richly coated with the bacteria, and both station 1 of Aldersey ditch and the Pasturefields ditch were covered with an iridescent film of iron hydroxide and contained a suspension of precipitated iron oxide,

presumably surrounding photosynthetic organisms. Low redox potentials resulting from large amounts of organic matter would allow reduction of ferric iron, though it is unlikely that the brine is the source of the iron, as raw brine contains only 0.4 - 0.45 mgl^{-1}.

2. FAUNA

a. Diptera.

The approximate contribution of the Diptera to total number of species is given in table 31 and it is clear that a large proportion of the invertebrate fauna, particularly at the more saline sites, belongs to this order.

Although emergence traps were not used at Pasturefields or at Aldersey station 2, the abundance of dipterous larvae in the pond net samples confirmed that the order was represented least at these two sites but was increasingly important at progressively more saline sites.

Several of the species found in high salinities have been shown by previous researchers to be capable of hypo-osmotic regulation of their extra cellular fluids, whilst others, whose regulatory abilities are unknown, undoubtedly possess a considerable independence of their external environment. These include those species which are characteristic of harsh environments, including concentrated media such as sap and animal dung. The remaining Diptera, although more euryhaline than most limnobionts, were species which are normally found in freshwater.

i. Species with established powers of hypo-osmotic regulation.

At the sites investigated Ephydra riparia was obviously the most successful species in extremely high salinities. Prolific numbers of the larvae were found crawling in the mud at Lower Wych pit and tunnelling in the algal carpet at Aldersey Spore. The pupae either moored themselves to each other to form rafts or to the

Table 31. The contribution of the Diptera to the brine spring faunas.

Locality	% abundance of immature Diptera in pond net samples*	Number pond net samples	Number of trapping occasions	Number Diptera species	Number invertebrate species (tot.survey)	% dipterous species	Halobic index
Lower Wych Pit	99%	4	6	4	7	57	3
Aldersey Spore	98%	8	8	19	27	70	5
Aldersey ditch (station 1)	97%	8	8	23	41	56	6
Aldersey ditch (station 2)	11%	7	-	5	16	31	10
Shrewbridge	87%	9	7	31	50	62	8
Pasturefields	57%	3	-	13	29	45	18

*These are minimum estimates as Ephydridae and Ceratopogonidae were often too abundant to count. Although Oligochaetae were not counted, they were much less abundant.

algal mat, utilizing the curious excision in the hind segments.

Carpenter (1928) considers that all species of Ephydra are holeuryhaline and previous records of the genus indicate that it is universally characteristic of hypersaline waters. [Utah, six species (Aldrich 1912), E.'riparia' 209°/ooS (Schmidt 1913), Ephydra spp. 222°/ooS (Hesse,Alleė and Schmidt 1937), E.macellaria 50^{+}°/ooS (Beadle 1943a), Ephydra sp. 210°/ooS (Remmert 1955b)].

The physiology of the species was first investigated by Beyer (1939) who found that the internal medium was completely independent of external salinity fluctuations. Nemenz (1960) and Sutcliffe (1959) further demonstrated that larvae of E.riparia can elaborate urine considerably more concentrated than seawater, and the presence of the species at Lower Wych is therefore not exceptional.

Several species of mosquito are also renowned as hypo-osmotic regulators characteristic of high salinity waters, and two of these, Aedes detritus and Culex pipiens were taken during this investigation.

Aedes detritus was taken at both Shrewbridge and Aldersey station 1 and Culex pipiens was taken at Shrewbridge and Aldersey station 2. Both species are salt tolerant and A.detritus even shows a preference for saline sites for breeding, (Beadle 1939). C.pipiens is found in the range 1-15°/ooS in the Baltic (Remane and Schlieper 1958), although the experiments of Wigglesworth (1938) indicated a more restricted range. He found that C.pipiens could regulate its blood concentration in media up to 7.5°/ooS and that regulation broke down beyond that point. C.pipiens may therefore only regulate hyper-osmotically.

Aedes detritus, however, is undoubtably capable of hypo-osmotic regulation. Beadle (1939) recorded Aedes detritus from far more extreme salinities (10-100°/ooS, Algeria) and showed that not only could the species regulate blood chlorides and total osmotic

pressure in media of up to 60°/ooS, but it also had reduced anal papillae with a low permeability. Ramsay, (1950) demonstrated that larvae kept in seawater could produce urine with a concentration three times that of the blood by actively absorbing water in the rectum. It therefore seems probable that the species would be capable of inhabiting the Lower Wych pit once initial colonisation had occurred. The species was prolific at concentrations of 42°/ooCl (71°/ooS) in a brine settlement reservoir on the Holford brinefield, 32km away (personal observation), and this is probably the nearest colony. As prevailing winds are SW the probability of colonisation from either that site or the coast may be low.

Certain of the species of Chironomidae found at the brine springs have also been shown to be tolerant of high salinities, and although some are certainly able to regulate hypo-osmotically, others may just possess some mechanism at the cellular level whereby they tolerate high salinities.

Chironomus aprilinus, C.salinarius and Halocladius varians (=Cricotopus vitripennis) are all predominantly coastal species and were all found at Shrewbridge brine pool. Neumann (1961) demonstrated that C.salinarius regulates strongly, both hyper- and hypo-osmotically and suggests a range of 1-37°/ooS based on field records. Sutcliffe (1959) studied the salinity tolerance of C.aprilinus and H.varians. He found that C.aprilinus was capable of completing its life-history at 19°/ooS in the laboratory and although the species was permeable to water and salts it could tolerate twice the rise in blood osmotic pressure tolerated by most freshwater insects. H.varians has a greater tolerance of salinity and breeds in salinities greater than sea water. Sutcliffe concluded that this tolerance is principally achieved by a reduction in permeability (anal papillae are absent), though he suggests that the typical variety has developed a mechanism at the cellular level which assists in maintaining water-balance.

The presence of these coastal species at Shrewbridge indicates very effective dispersal, the nearest coastal marshes being over 30km away. Macan (1961) draws attention to the wide dispersive ability of chironomids which he reports have been taken in nets over the sea 20km from the coast.

ii. Salinity resistant species characteristic of harsh environments. Although Ephydra riparia was the most widespread representative of its family, other members were taken at Shrewbridge, Aldersey and Lower Wych. The family is well-equipped for life in concentrated media and bear spiracles on the hind segments with siphons of various lengths which assist in respiration.

Of the smaller Ephydridae, apparently only Scatellini have been recorded from saline waters and in this respect the record of Atissa pygmaea at salinities of up to 69.5°/ooCl (116.8°/ooS) is new. Thienemann (1913) found Scatella stagnalis at just over 112°/ooS and Remmert (1955b) considered it a holeuryhaline species. Hydrellia griseola, H.modesta and S.stagnalis have all been taken from sewage sludges (Green 1970) and their presence in the brine pools is therefore not surprising.

Other families characteristic of dung and concentrated media were found in the brine and these included Sepsidae, Psychodidae, Sphaeroceridae, Scatophagidae, Ceratopogonidae, Tabanidae and Dolichopodidae. Like the Ephydridae many of the larvae of these families have reduced their permeability to the external medium, for example by having spiracles on raised siphons and surrounded by water-repellent hairs.
Several of the genera and species found at the brine pools have been previously recorded from saline habitats. The genus Leptocera, which was represented at Lower Wych pit, was recorded by Thienemann (1913) in hypersaline waters in Westphalia (L.limosa (Fallén) at 112°/ooS and L.fontinalis (Fallén) at 102°/ooS). Thienemann (op.cit) also took Scatophaga litorea (Fallén) at 23°/ooS in Westphalia. Schwarz (1891) reported finding larvae of

Tabanus sp. in the shallow pools of the salt flats around Great Salt Lake, Utah. Thienemann (1915) names six species of Ceratopogonidae from the Westphalian salines and Remmert (1955b) names another three species as being holeuryhaline, and two others as tolerating salinities up to 15°/ooS. Thienemann (1913) took Psychoda phalaenoides at 115°/ooS in Westphalia. In the present study many species of Psychodidae were taken at Aldersey and Shrewbridge including P.phalaenoides, and yet none were recorded from the hypersaline water of Lower Wych pit. Some Dolichopodidae are known to frequent seaweed and Colyer and Hammond (1968) report that they have taken Campsicnemus sp. in company with Ephydridae. Oldroyd (1964) reports that both Campsicnemus sp. and Ephydridae feed on insects trapped in the surface films of water, or in algae, where these reach the surface. It is not surprising, then, that Dolichopodidae were recorded from Shrewbridge, Aldersey Spore and Aldersey station 1. Brock et al (1969) report that Dolichopodidae are predatory on the eggs and larvae of Ephydridae.

Other families which are characteristic of concentrated media, albeit not dung dwellers, and which were found in the brine pools, include the Syrphidae, the Tipulidae, the Stratiomyidae, and the Empidae which includes some coastal species. Syrphid larvae, commonly known as rat-tailed maggots were taken at both Aldersey Spore and Aldersey station 1. Two species, Lejops vittata Meigen and Eristalinus aenus (Scopali) are coastal, and the latter was recorded in hypersaline water by Thienemann (1913). Schwarz (1891) records the emergence of a green syrphid fly from the mud of the salt flats around Great Salt Lake and Beadle (1943a) took numerous larvae of Eristalis sp. at Lake Katwe in E.Africa which is a saturated salt lake. Tipulid larvae also inhabit brackish and saline habitats (Oldroyd 1964). Thienemann (1915) recorded a member of the family in high salinities in Westphalia and Remmert (1955b) considered Symplecta stictata Meigen to be holeuryhaline.

The only representative of Stratiomyidae taken in the present study was Nemotelus notatus which was taken at Lower Wych pit

(apparently in lower numbers than recorded by Burke (1942b)). Although the physiology of the species has not been investigated, the distribution of the species suggests that it may have osmoregulatory abilities similar to Ephydra riparia. Thienemann (1913) found the species at over 104°/ooS in Westphalia and Remmert (1955b) suggests that it is holeuryhaline. Thorpe (1927) found Nemotelus sp. in coastal pools and unidentified Stratiomyidae in Owen's Lake, California, at 15.9 - 213°/ooS (Thorpe 1931). Howes (1937) also recorded Nemotelus sp. from Essex at 21-28°/ooS in New England Creek.

iii.Essentially freshwater species. Those species recorded in the brine springs which must be considered essentially freshwater species belonged mainly to the Chironomidae, though some Chaoboridae were taken.

The two individuals of Chaoboridae taken at Aldersey Spore are of interest as larvae of this family breath entirely by absorption over the integuement (Colyer and Hammond, 1951) and a corresponding high permeability to water and salts would be expected. The larvae were taken in January and February at salinities of between 9,180 and 9,300 mgl^{-1}Cl (15,420 - 15,620 mgl^{-1}S), and apart from a Canadian record of Chaoborus (Corethra) sp. at 7,870 mgl^{-1}S (Rawson and Moore, 1944), this appears to be the only record of the family in mixohaline water.

Freshwater Chironomidae were found at every site in the present study except Lower Wych pit, Pasturefields having the greatest variety. While most species appeared to be only slightly euryhaline, Cricotopus sylvestris, Chironomus riparius and Procladius sagittalis were euryhaline to the third degree. The distribution of Cricotopus sylvestris is noteworthy in that it was absent from Aldersey Spore and yet present in the adjacent ditch. As Remmert (1955b) found the species only in the range 0-10°/ooS in the Baltic, it seems that its tolerance of salinity may be insufficient to permit its survival in Aldersey Spore. Chironomus

riparius showed a similar distribution in the brine pits, and although abundant in Aldersey ditch and the less saline sites, was infrequent in Aldersey Spore. This is in accord with its distribution in the Trent and Mersey Canal and in Crow Brook where peak abundance occurred between 2°/oo - 3.5°/ooCl (3.4°/oo - 5.9°/ooS). In Crow Brook larvae were never found at salinities in excess of 9°/ooCl (15.1°/ooS), and allowing for drift, this does not greatly exceed the range recorded by Remmert (1955b), and Sutcliffe (1959), who considered that it did not exceed salinities of 8-10°/ooS. Procladius sagittalis was yet another species found abundantly at Aldersey station 1 and at the Shrewbridge pool and yet only occasionally in Aldersey Spore, and the distributions of the three species suggest that freshwater chironomids are near the limits of their tolerance at 9-10°/ooCl (15-16.8°/ooS).

Additional support for this hypothesis comes from previous observations on salt-marsh faunas. Sutcliffe (1959) concluded from physiological investigations of some members of the fauna at Seaton Sluice that a permeable insect cannot survive in external concentrations greater than 10°/ooS unless the tissue cells become adapted to an increased osmotic pressure of the blood. Butler & Popham (1958) arrived at a similar conclusion from their survey of the aquatic insect fauna of the Spurn Peninsula. Their species lists indicate that 25°/o sea water (8.4°/ooS) was critical for many freshwater insects. It is hardly coincidence that the isosmotic point for the majority of freshwater invertebrates lies between 5-15°/ooS (Beadle 1959 , Bayly, 1972), and it seems probable that an explanation for the great differences in the abundance of P.sagittalis, Chironomus riparius and Cricotopus sylvestris between Aldersey Spore and the adjacent station 1, may be sought in terms of osmotic tolerance.

Adaptations of Diptera. It is apparent then from the preceding discussion that the Diptera play a dominant role in the colonization of inland saline waters and their predisposition

for this type of habitat originates from several factors:

(i) The aquatic Diptera have evolved from terrestial ancestors which had overcome the problems of dehydration, by the possession of an impermeable cuticle,by restricting water loss in the urine and, by the ability of cells to adjust to changes in body fluid concentration. Sutcliffe (1959) discusses the ability of several Diptera to live in salt water in terms of their possession of one or more of these mechanisms. Chironomus aprilinus appears to be permeable but possesses the ability to regulate the electrolyte fraction of the blood. Halocladius varians var halophilus relies almost entirely on impermeability to the external medium and regulation of the water content in the tissue cells is scarcely necessary. The typical form of the species utilizes both impermeability and the ability of the tissue cells to maintain water balance and can tolerate higher salinities. The third mechanism, elaboration of urine hypertonic to the medium,is seen in Aedes detritus and this is combined with extreme impermeability in Ephydra riparia. Potts and Parry (1963, p.313) support the view that the ability of the rectal glands to conserve water, which is so important for life on dry land, has been instrumental in permitting some species to live in hypersaline waters.

(ii) The ability to utilise atmospheric air for respiration has been retained by many dipterous larvae, such as those which possess siphons e.g. Syrphidae and Ephydridae, and this permits independence from the external medium. Other species have developed a truly aquatic respiration which allows penetration into waters with turbulent surfaces. Mixohaline species have increased impermeability by the reduction of the anal "gills" e.g. Aedes detritus and Halocladius varians.

(iii) In marine habitats the ability of the pupae of Diptera to hatch directly on to the free water surface has provided an advantage over other orders (with the exception of some Ephemeroptera & Trichoptera) which require a solid substrate, (Remane and Schlieper 1958).

(iv) Impermeability of the eggs, which is essential for terrestial life has been retained by the aquatic Diptera.

(v) Dispersal is achieved by an aerial stage of the life-history and individuals are therefore well equipped to colonize isolated saline waters, in contrast to those invertebrates which complete their life-history in water.

b. Other Insecta

Although Diptera were the predominant group, members of other orders were present, particularly at the localities with less extreme salinities.

Collembola. These were found at Pasturefields, Shrewbridge and in Aldersey ditch and appear to be tolerant of moderately saline conditions. After the catastrophically high salinities in January 1972 at Shrewbridge, corpses of collembolans were collected which suggests that they are unable to tolerate 33^{o}/oo Cl (55^{o}/ooS). The absence of the group at Aldersey Spore and Lower Wych pit may also indicate a limited tolerance of salinity.

Thysanoptera and Homoptera. These appeared occasionally in the emergence traps and their occurrence must be taken as incidental as the majority of thrips and aphids are plant feeders.

Hemiptera. Three freshwater species of Hemiptera were recorded, Notonecta glauca and Sigara dorsalis at Pasturefields and Gerris thoracicus at Shrewbridge. Staddon (1963) noted that Notonecta glauca was restricted to freshwater in coastal areas but was unable to demonstrate an osmoregulatory reason for this.

A breeding population of the mixohaline species, Sigara stagnalis was also found at Shrewbridge with 10 nymphs being taken from 7.1^{o}/oo Cl (11.9^{o}/ooS) in September 1972, and a female of the same subgenus, Sigara Halicorixa (comprising S.stagnalis and S.selecta) was taken at Lower Wych. Unfortunately there is no reliable

characteristic for the separation of females of the two species of S.Halicorixa. The laboratory investigations of Claus (1937) demonstrated that S.stagnalis (=S.lugubris) was perfectly homoiosmotic in external media of up to 14°/ooS at which point he claims hypotonic regulation commenced and continued up to 20°/ooS. In the field, Lansbury (1954) has taken S.stagnalis at 20.75°/ooS at Sandwich and Savage (1971) has taken the species from around 15°/ooS in the saline flashes of Cheshire. The distribution of the species on the Holford brinefield will be discussed in the next chapter. The subgenus S.Halicorixa is not normally found in freshwaters, and therefore the Lower Wych specimen must have originated either from the Shrewbridge population 21km away, or more probably from a distance of over 32km, either from the coast or the flashes and brine ponds of the Northwich and Sandbach area.

Neuroptera. Larvae of Sialis lutaria were fairly common at Pasturefields in salinities of nearly 1,700 mg l^{-1}Cl, (2860 mg l^{-1}S) and this is not unexpected in the light of Shaw's investigations (1955a, 1955b,) which established that larvae will survive salinities of about 4.8°/ooS for at least two months by controlled drinking of the external medium.

Ephemeroptera. These were also only taken at Pasturefields, where they were represented by Centroptilum pennulatum in salinities of up to nearly 1,700 mg l^{-1}Cl. It has already been mentioned (Chapter III) that this order is considered strictly stenohaline and this species must therefore be included with Baëtis rhodani as being exceptionally tolerant.

Trichoptera. Caddis were only represented by Limnephilidae, and at Aldersey station 1 and Shrewbridge these were positively identified as Limnephilus affinis, the species found at Seaton Sluice by Sutcliffe (1959) in concentrations reaching that of sea water. In the laboratory he found that tolerance of salinity broke down around 28°/ooS. The distribution of the species in the Cheshire brine springs is therefore not exceptional.

Coleoptera. Several species of Coleoptera were found, though only three species at Aldersey Spore and one at Lower Wych. Although Balfour-Browne found several mixohaline species inland in Cheshire (G.Foster, pers.comm), Enochrus-4-punctatus was the only one of these found in the present survey. The specimens appeared to be intermediate between the type form and var.halophilus but unfortunately the Shrewbridge site was destroyed before further specimens could be collected.

Haliplus lineatocollis was found at the two most saline stations, though it may have been a transient visitor, as all but one of the beetles taken from Aldersey Spore were taken on the emergence traps and were obviously leaving the water.

Although Sutcliffe (1959) found both Hemiptera and Coleoptera at salinities reaching that of sea water, they were rare above $10^{o}/_{oo}S$ and he considered that as transpiration rates through the cuticle are extremely low, this scarcity is the result of preference for freshwater, not inability to tolerate saline water. He noted in particular that Agabus bipustulatus immediately migrated from the salt-marsh at the onset of high spring tides and speculated that chemoreceptors would be likely to evolve in a species performing regular migrations and coming into contact with a wide range of aquatic environments.

Hymenoptera. Small specimens were taken frequently in the emergence traps at Lower Wych, Aldersey Spore and station 1, and rather less frequently at Shrewbridge. Although unidentified, it seems very probable that these are chalcids of the family Pteromalidae,which parasitises Ephydra riparia (Burke 1942b). Green (1970) took seven species of this family and 21 other species of Hymenoptera from sludge drying beds and considered that many of these were parasitic on dipterous larvae in the sludge.

c.Other invertebrates.

The lower phyla were only well represented at the less saline sites.

Platyhelminthes. Polycelis tenuis was found at nearly 1.7°/ooCl at Pasturefields, at a rather higher salinity than it was recorded in Crow Brook.

Oligochaetae. Species were not normally identified, though the littoral species Lumbricillus reynoldsoni was the species found in Aldersey Spore.

Mollusca were only recorded at the two least saline sites. Potamopyrgus jenkinsi was prolific at Aldersey station 2 which had a salinity range 1,580 - 5,460 mgl^{-1}Cl, similar to that of station 4 of Crow Brook (i.e. 160-4,000 mg l^{-1}Cl), which was where it reached peak abundance at that locality. Only a single specimen of P.jenkinsi was taken at Pasturefields where Lymnaea pereger was the dominant mollusc, being found in salinities up to 1,740 mg l^{-1}Cl. The range of the species in the Trent and Mersey Canal had not indicated whether it was stenohaline or euryhaline 1°, and this record confirms a degree of euryhalinity. Planorbis sp. is also apparently reasonably tolerant, having been recorded up to 1,800 mg l^{-1}Cl at Aldersey station 2.

Crustacea. Gammarus pulex and Asellus aquaticus were taken at the two low salinity sites, though two specimens of A.aquaticus were found in the saline stretch of Aldersey ditch. The greatest salinity at which G.pulex was found was 3,080 mg l^{-1}Cl (5,170 mg l^{-1}S) whereas A.aquaticus was taken up to 4,500 mg l^{-1}Cl (7,560 mg l^{-1}S). These compare well with their ranges in Crow Brook where G.pulex was becoming scarce at mean salinities of 1,600 mg l^{-1}Cl, but was present up to mean salinities of 3,100 mg l^{-1}Cl. A.aquaticus was declining in abundance around 2,000 mg l^{-1}Cl and was also scarce at mean salinities of 3,100 mg l^{-1}Cl. The high variability undoubtedly accounts for the rather more restricted ranges of the two species in Crow Brook.

Among the lower groups, all species found at Pasturefields must be considered to be at least euryhaline 1°. Occasional records of

species at Aldersey station 2 cannot necessarily be taken to indicate euryhalinity 3° as a full frequency distribution is needed to indicate the successful range of a species. Asellus aquaticus and Potamopyrgus jenkinsi are undoubtedly euryhaline 3°, and the evidence from Crow Brook that they are also halophye cannot be contradicted. Limnephilus affinis probably also occupies a halophye position, though more extreme, and is obviously very successful in the more saline part of Aldersey ditch where it is probably predatory, and may account for discrepancies between abundance of larval Chironomidae and abundance of emergent imagines. Most of the Diptera are strongly euryhaline though the salinity boundary between Aldersey Spore and the adjacent ditch at around 15°/ooS appears to be a critical one for several species. Many of the Coleoptera and also Procladius sagittalis and Chironomus riparius show great differences in abundance between the two sites, although they are only separated by 2m of earth at the nearest point.

Among the Diptera,the dung feeders, Dolichopodidae, Psychoda, Scatophaga, Sepsidae and Ephydridae are all extremely euryhaline and possibly show a preference for brackish localities. Ceratopogonidae, Tabanidae, Syrphidae and Stratiomyidae are also associated with concentrated media and are obviously more tolerant than the permeable species of Diptera. Ephydra riparia, Atissa pygmaea, Leptocera sp. and Nemotelus notatus all approach holeuryhalinity.

Sigara (Halicorixa) spp, Halocladius varians, Chironomus aprilinus and C.salinarius are all true mixohaline forms and their presence in these localities is evidence of a dispersive range which is probably in excess of 32km.

SUMMARY

1. Four localities where natural brine reaches the surface were investigated and covered the salinity spectrum from freshwater to 69.5°/ooCl (116.8 °/ooS).

2. The fauna is discussed systematically. Among the non-insectan groups, only Lumbricillus reynoldsoni crossed the 10°/ooCl boundary although several species showed euryhalinity to the second and third degrees.

3. Of the non-dipterous insects, only a few Coleoptera were found at very high salinities and these appeared to prefer less extreme concentrations. The caddis larva, Limnephilus affinis, was also very tolerant and found in salinities up to 9°/oo Cl (15°/ooS).

4. Members of the Diptera dominated the fauna, especially at the more saline sites. Chironomidae of freshwater origin appeared less tolerant of high salinities than the more impermeable dung feeding Diptera. At least four species of the Diptera appeared to be holeuryhaline.

5. At least one coastal species of corixid and three coastal species of Chironomidae were recorded and their occurrence at inland locations indicates a capacity for widespread dispersal.

CHAPTER VI

THE CORIXIDAE OF THE HOLFORD BRINEFIELD.

INTRODUCTION

Apart from the work of Savage (1971), the Corixidae of brackish and mixohaline waters have received little attention in Britain. Savage investigated a series of flashes, the salinity of which ranged from that of freshwater to 16.6°/ooS). Ten species of corixids, including the mixohaline species Sigara stagnalis Leach, were recorded and Savage was able to relate the distribution of five of these to salinity. S.stagnalis has been recorded all around the British coast (Southwood & Leston 1959, Walton 1943, Brown 1943) and Lansbury (1954) recorded a breeding population in a salinity of nearly 21°/ooS on the Kent coast. Claus (1937) investigated the osmoregulatory ability of the species in the laboratory. One other strictly mixohaline corixid occurs in Britain, S.selecta (Fieber) and these two comprise the subgenus Sigara Halicorixa. S.selecta is rare in Scotland and absent from Ireland and Wales,yet has been recorded in nearly every coastal county of England (Southwood and Leston 1959). The ecology of the species has, however, been rather neglected, although Macan (1954) found it co-existing with S.stagnalis in one of the two salt-marsh ponds sampled in his extensive survey of corixid habitats. Butler and Popham (1958) also recorded the two species together on the Spurn peninsula, though Howes (1939) found S.selecta alone in an Essex lagoon. Macan (1939) recorded a single specimen of the species on the wing in Cambridge.

On the continent Lindberg (1936, 1948) recorded nine species of corixid from the Baltic, though only two, S.stagnalis and S.striata (Linnaeus) (thought to be the S.dorsalis of British workers) exceeded salinities of 6°/ooS. Van der Velde and Polderman (1974) studied the fauna of four brackish sites with salinities up to 12.7°/ooS in the polder lands of Zeeland where six species of corixids, including S.stagnalis occurred.

Description of the sites.

The ponds of the Holford brinefield (fig.22) are situated within an area 1700m x 1300m amidst a rectangular network of boreholes. The ponds had received varying amounts of brine from spray, pipe and valve leakages and from run-off when pressurised air and brine were vented from boreholes to permit inspection of the cavities.

The ponds ranged in size from Pond 7 which had a diameter of about 14m, to Pond 6 which consisted of two linked ponds and was approximately 18m x 40m. All of the ponds were set in open grassland which was grazed by cattle, except for Pond 2 which was in a small copse of sycamore (Acer pseudoplatanus L), alder (Alnus glutinosa (L)) and hawthorn (Crataegus monogyna Jacquin) and Pond 3 which was rather more open, though overhung by occasional alder trees and hawthorn bushes. Ponds 1 and 5 had bushes along one bank and Pond 9 was situated against a sparse hawthorn hedge. The remaining ponds were in open fields. All the ponds were shallow, none exceeding 1m in depth, and they contained no macrophytes. Pond 11 was fringed by floating rafts of grass and Pond 8 contained Enteromorpha intestinalis. Intermittent visits were also made to two small settlement reservoirs about 1km away (R_1 and R_2), which were used for waste brine.

METHODS

The ponds were visited regularly over the period from January 1973 to March 1974 and with increased intensity as the work load at other sites diminished. The sampling programme is presented in table 32. Samples of surface water were taken in the usual manner and analysed for chlorides, conductivity and pH. Water samples were analysed for calcium and magnesium on 24th February and 24th March 1974. Specimens of Corixidae were taken by sweeps of a Freshwater Biological Association pond net. Sampling was continued for 5 minutes at each of three sampling points around a pond, and returned to the laboratory for counting and identification of corixids. Other invertebrates taken in samples were recorded, but were not normally identified to species level.

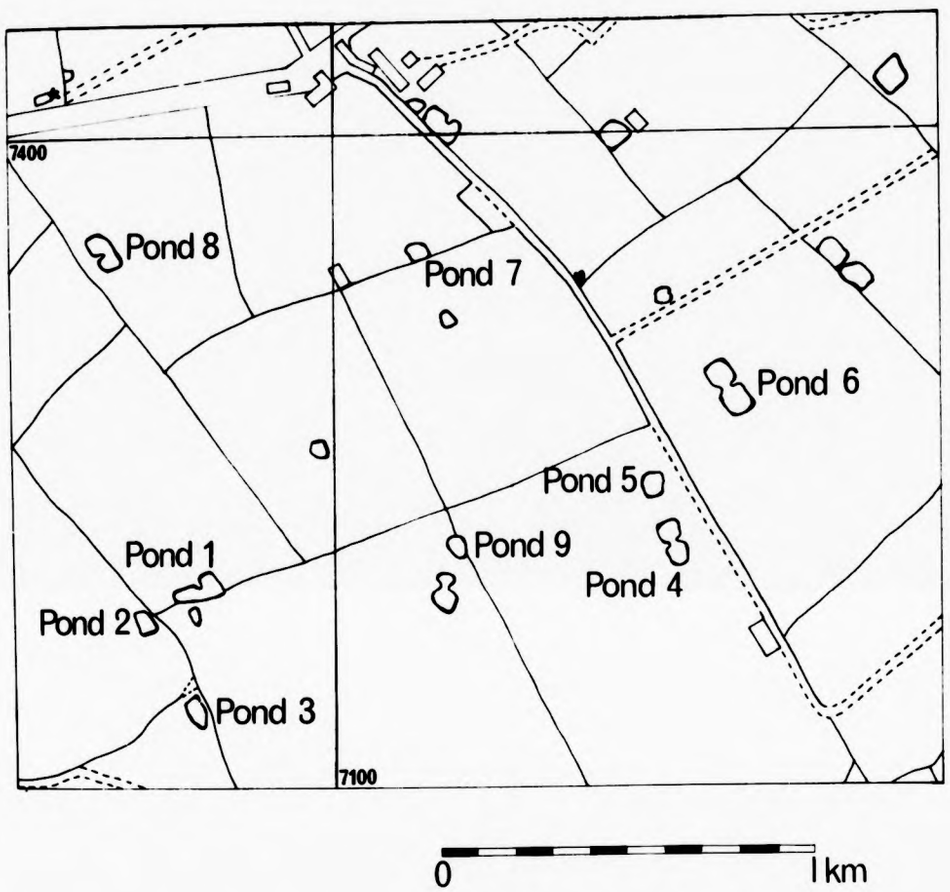


Fig. 22. Location of the ponds of the Holford Brinefield.

Table 32. Sampling programme for the Holford ponds.

Date	17. 8.72	12. 9.72	19. 1.73	16. 2.73	20. 3.73	24. 7.73	9.10.73	28.10.73	30.11.73	4. 1.74	24. 2.74	27. 3.74
Station number												
1			x	x	x	x	x	x	x	x	x	x
2			x	x					x	x	x	x
3				x					x	x	x	x
4				x	x	x	x	x	x	x	x	x
5				x	x	x	x	x	x	x	x	x
6				x	x	x	x	x	x	x	x	x
7						x	o	x	o	x	o	o
8							x	x	x	x	x	x
9							x	x	x	x	x	x
R1	x	x			x			x				
R2	x	x			x	x						

x faunal and water samples
o water samples only

RESULTS

The chemical parameters of the ponds are presented in table 33, and the numbers of the various species of corixids from each pond and for each sampling occasion are listed in table 34. Pond 9 had the greatest diversity of corixids, but five of the species were only recorded in low numbers and only in October, during anticyclonic conditions, and are probably migrants. Sigara stagnalis was only recorded in very low numbers in this pond and is probably not a permanent resident. Records of nymphs are presented in table 35.

A comparison of the corixid faunas of the ponds was made using the index of similarity (I), where $I = [\sum_{i=1}^{n} a_i b_i][\sum_{i=1}^{n}(a_i^2 b_i^2)]^{-1}$ and a_i is the percentage of individuals of a given species at one site and b_i is the percentage of that species at the site with which it is being compared. These percentages are used in the computation for each of the n species listed for the combined sites. Numbers of males only of S.Halicorixa sp. were included in the analysis as the females of the two species could not be reliably separated. The indices were then used to construct a dendrogram (fig.23) after the method of Mountford (1962) using the general formula $\frac{1}{lm} \sum_{k=1}^{l} \sum_{j=1}^{m} . I . (A_k B_j)$ to compare group A and group B where l and m are the number of sites comprising those groups respectively. Population numbers and numbers of samples for each site were generally high and the method could therefore be considered reliable. Ponds 3 and R2 were not included in the analysis as the former contained only one species, Hesperocorixa sahlbergi, and the latter yielded only one specimen during the whole study.

The analysis reveals that the ponds fall into three groups. Ponds 7, 8 and R_1 are very closely related with indices of affinity $\geqslant$ 0.98 and Pond 6 is loosely associated with this group. Ponds 4 and 5 comprise the second group with which Pond 9 has an affinity of 0.58, and Group C is comprised of two freshwater ponds, 1 and 2. The sequence from R_1 to Pond 2, as presented in the dendrogram (fig.23), corresponds to a sequence of decreasing

Text continues on P.174.

Table 33. Salinity, hardness and pH records for the Holford ponds.

Pond number	Salinity mg l^{-1} Cl		Hardness (mg l^{-1})			
	Mean	Extreme range	Ca	Mg	Total	pH ± s.d.
1	830	260- 1,850	98	20	118	7.3 ± 0.6
2	920	159- 3,420	92	21	113	6.9 ± 0.4
3	96	72- 106	105	24	129	7.3 ± 0.2
4	1,550	804- 2.470	68	13	81	7.4 ± 0.2
5	1,430	300- 2,610	70	16	86	7.1 ± 0.5
6	4,970	2,620- 8,280	85	13	98	7.5 ± 0.7
7	1,360	530- 2,600	89	22	111	7.9 ± 0.3
8	20,250	14,360-29,640	180	42	222	7.1 ± 0.4
9	185	26- 440	76	22	98	7.6
R1	18,700	5,000-40,300	-	-	-	7.8
R2	28,800	13,100-59,100	-	-	-	6.4 ± 0.7

Table 34. Numbers of corixids taken at each site.

	Pond number											
	1	2	3	4	5	6	7	8	9	R1	R2	Total
Callicorixa praeusta (Fieber)	3			2	1		1		5*			11
Corixa punctata (Illinger)							1		50			51
Hesperocorixa sahlbergi (Fieber)	2	9	827	1	2				2*			843
Sigara dorsalis (Leach)	10	86							1*			97
S.distincta (Fieber)									1			1
S.falleni (Fieber)				1	1							2
S.lateralis (Leach)	2	1		105	383	11			151			653
S.nigrolineata (Fieber)	1											1
S.concinna (Fieber)				6	18	1			42			67
S.limitata (Fieber)									1*			1
S.selecta (Fieber)	3			109	191	162	8	12		3		488
S.stagnalis (Leach)	10			23	31	219			8			291
S.Halicorixa sp.	20			497	568	679	1	55	25	5	1	1851
Total	51	96	827	744	1195	1072	11	67	286	8	1	4357

*four of the species in pond 9 were only recorded in October.

Table 35. Nymphs of Sigara Halicorixa spp. taken on the Holford brinefield.

Pond Number.	Date	Number of nymphs	Salinity (mg l^{-1} Cl)
4	24. 7.73	85	2,210
5	24. 7.73	131	2,610
5	24.10.73	5	1,870
6	24. 7.73	160	2,750
6	24.10.73	9	8,140
R1	17. 8.72	1*	4,940

*probably S.selecta

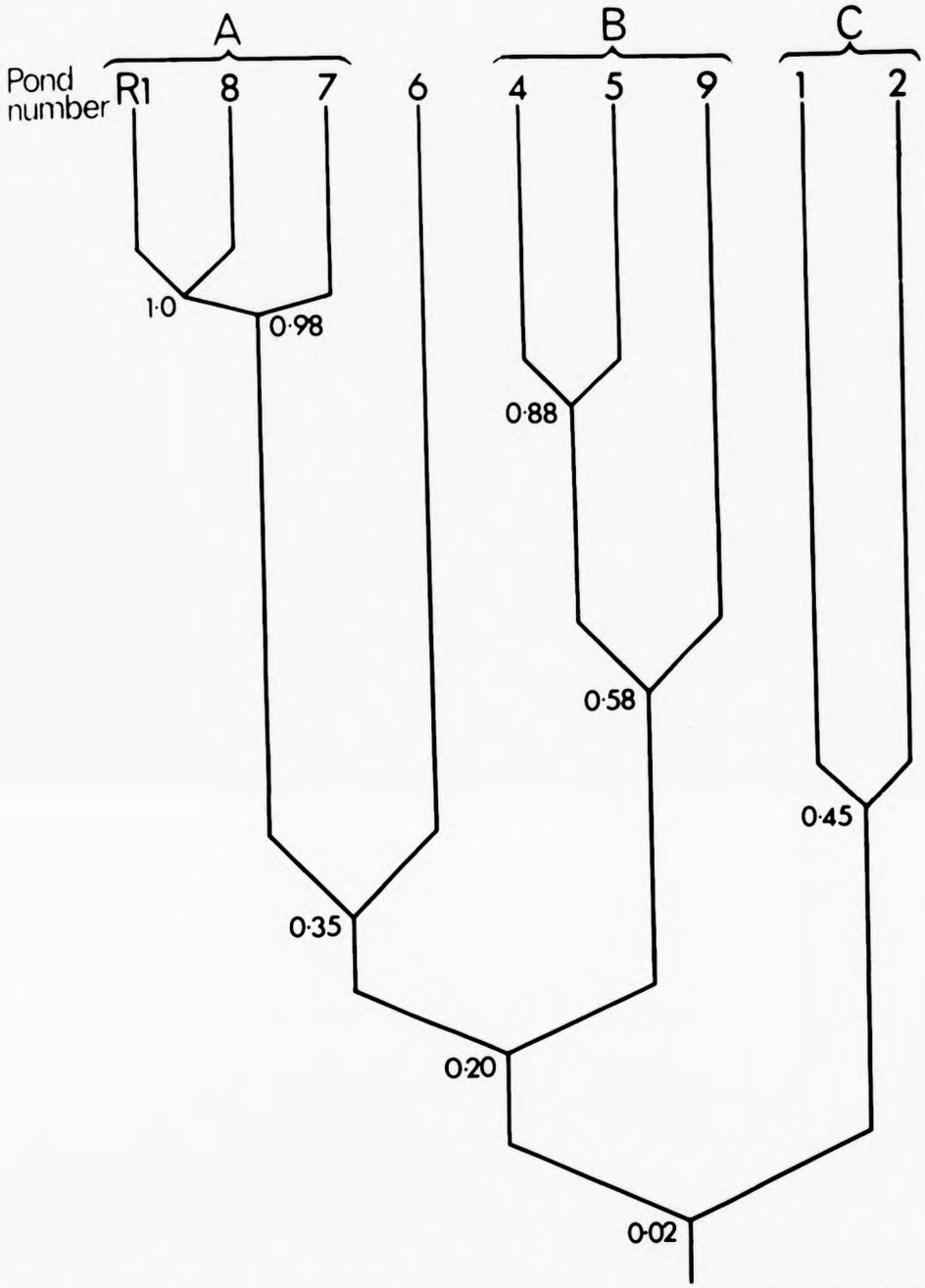


Fig. 23. Dendrogram showing the relationship between the Holford ponds based on an analysis of their Corixid faunas.

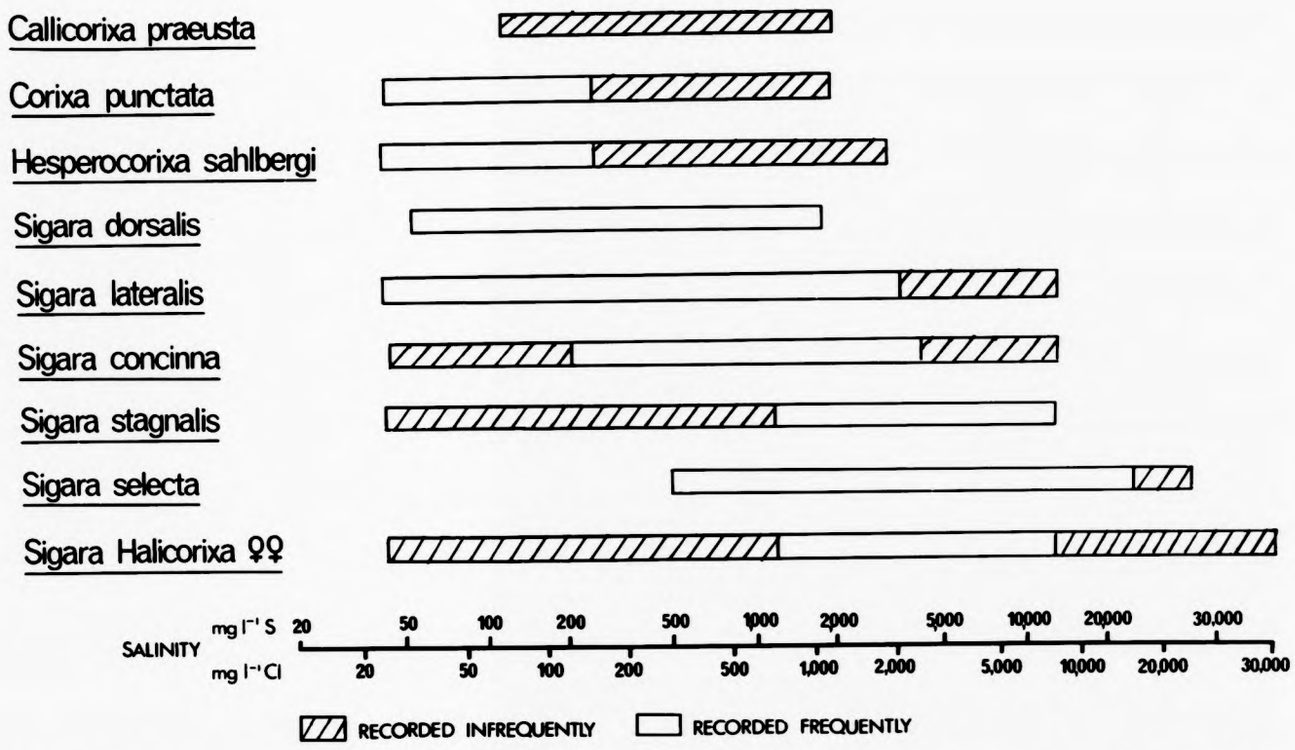


Fig. 24. Range of the dominant species of Corixid on the Holford Brinefield in relation to salinity.

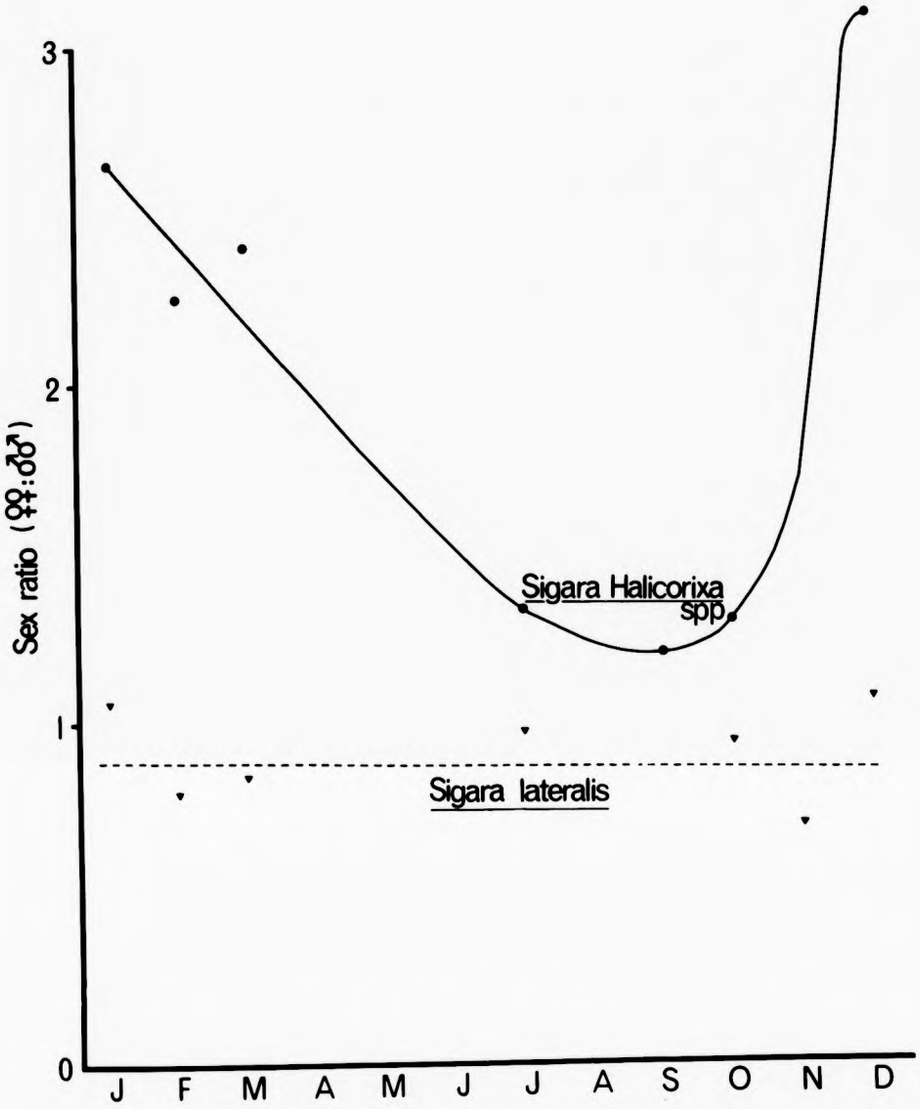


Fig. 25. Seasonal changes in the sex ratio of Sigara Halicorixa spp. and S. lateralis on the Holford Brinefield.

salinity, ponds 7 and 9 excepted (the corixid faunas of which have unexpectedly high affinities with more saline habitats).

The range of salinity over which the common species were recorded is represented in figure 24 and those parts of the range where individuals occurred infrequently are cross-hatched. Sigara concinna, S.stagnalis and S.selecta were all more abundant in moderate salinities than in freshwater. S.lateralis was commonly found in waters of up to 2,600 mg l^{-1}Cl (4,370 mg l^{-1}S) and S.dorsalis in waters up to 1100 mgl^{-1}(Cl),(1850 mgl^{-1}S). Coefficients of association (c) could not be computed for most combinations of species as the technique is invalid where species are either rare or very common (Southwood,1966,p328). It was, however, possible to compute values for the association of S.lateralis with the three halophye species using the method of Cole (1949) and these are presented in table 36.

Table 36.
Coefficients of association between S.lateralis and three halophye Corixid species.

		S.concinna	S.stagnalis	S.selecta
S.lateralis	Coefficient of assn.	0.69	0.56	0.37
	χ^2	6.84	15.8	10.66
	Significance of assn.	$p < 0.01$	$p < 0.001$	$p < 0.01$

Seasonal changes in the sex ratio of S.Halicorixa sp during 1972 were calculated and are represented in figure 25. Unfortunately no samples were taken over the period April-June owing to pressure of work elsewhere. Large numbers of individuals were involved in the computation of each point and reliability is high. Although

there were more females than males all year round, a significant feature of the curve is that the ratio of females to males is greater than 2 for the period December to March, and exceeds a value of 3 in December. In July, September and October the ratio was at its lowest, having a value just greater than 1. The sex ratio of S.lateralis during the same year was also calculated, though as numbers were lower no attempt has been made to draw a curve through the points. The ratio for this species remains in the region of 1 all year round.

Other species associated with the Corixidae in the ponds are listed in table 37 and ponds 8 and R_1 in particular have faunas very tolerant of high salinity.

DISCUSSION

1. Species present and their habitat preference.

Of the twelve species of corixid taken from the ponds of the brinefield, four were rare (Sigara distincta, S.falleni, S.limitata and S.nigrolineata) and these were probably migrants. The distributions of the other species are ranked in order of their degree of penetration into saline waters in figure 24. Callicorixa praeusta was only taken infrequently but is included as Walton (1943) occasionally found it in rhynes in Somerset. Hesperocorixa sahlbergi and Corixa punctata had a restricted range but this was probably due to specific habitat preference, as the former was only recorded once outside pond and the latter rarely at sites other than pond . C.punctata prefers eutrophic ponds (Macan 1954) and H.sahlbergi is highly characteristic of small ponds that are constantly floored with leaf litter and with a restricted fauna (Macan 1954, Southwood and Leston 1959, Walton 1943), a characteristic for which the last named author condemned it as a species of little interest. The range of Sigara dorsalis, to salinities of 1,100 $mgl^{-1}Cl$ (1,850 $mgl^{-1}S$) is probably an accurate indication of a preferred range, and although it was only taken regularly from ponds 1 and 2 it was not found there on the occasions when

Table 37. The fauna of the Holford Ponds.

		Station number.										
		1	2	3	4	5	6	7	8	9	R_1	R_2
Platyhelminthes												
Tricladida	Polycelis tenuis Ijima									x		
Annelida												
Oligochaeta	Lumbricillus reynoldsoni Backlund										x_4	
	unidentified Enchytraeidae				x	x						
Mollusca												
Gastropoda	Limnaea (Radix) pereger (Müller)											
	L. (Galba) truncatula (Müller)	x								x		
Crustacea												
Isopoda	Asellus aquaticus	x								x		
Amphipoda	Gammarus pulex (Linnaeus)		x									
Insecta												
Odonata	Ischnura elegans (van der Linden)	x					x					
Hemiptera	Notonecta glauca Linnaeus									x		
	N. viridis Delcourt									x		
Trichoptera	Limnephilus affinis Curtis	x			x	x	x	x	x_1			x_2
	L. flavicornis (Fabricius)	x	x									
	L. stigma Curtis	x	x							x		
	L. politus Mc Lachlan			x								
	unidentified Limnephilidae											
Coleoptera	Haliplus immaculatus Gerhardt				x							
	H. lineatocollis (Marsham)		x			x				x		
	Helophorus grandis (Linnaeus)	x			x	x						
Diptera	unidentified larvae				x	x						
(Culicidae)	Aedes detritus (Haliday)				x				x_1		x_5	
(Chaoboridae)	Chaoborus sp.								x_1	x		
(Ceratopogonidae)	unidentified				x	x						x_3
(Chironomidae)	Procladius sagittalis (Kieffer)	x			x	x	x			x		
	Cricotopus sp.	x									x_4	
	Glyptotendipes sp.	x									x_4	
	Chironomus sp.		x				x	x	x_1		x_4	
(Stratiomyidae)	Nemotelus notatus Zetterstedt								x_1			x_3
(Ephydridae)	Ephydra macellaria Egger										x	
	E. riparia Fallèn				x				x_1			x_3

x = species present

x_1 = at 14,400 $mgl^{-1}Cl$

x_2 = at 13,100 $mgl^{-1}Cl$

x_3 = at 59,000 $mgl^{-1}Cl$

x_4 = at 8,500 $mgl^{-1}Cl$

x_5 = at 40,000 $mgl^{-1}Cl$

their salinity exceeded that value. Savage (1971) recorded the species regularly in three of the Sandbach flashes up to 1,250 $mgl^{-1}Cl$ (3,000 $mgl^{-1}S$).

S.lateralis was more widely distributed, both between ponds and over the salinity spectrum,and was common in ponds 4, 5 and 9, and present in small numbers in pond 6. It is probably a good indicator species as it migrates readily and is well known for rapidly colonizing new localities and deserting old ones as their suitability for the species changes (Southwood and Leston 1959). These authors consider that the preferred habitats are brackish pools or ones fouled by dung.

Walton (1943) found S.lateralisin salinities up to 265 $mgl^{-1}S$ all of which were shallow with no macroseopic plant life. This distribution corresponds well with its distribution in the Sandbach flashes where Savage (1971) found it commonly only in Moston and Crabmill flashes where total salinities reached as much as 572 $mgl^{-1}S$. Van der Velde and Polderman (1974) found the species occurred widely in brackish water and recorded it at salinities in excess of 2,500 $mgl^{-1}S$. In the present study S.lateralis was abundant up to salinities of 2,600 $mgl^{-1}Cl$ (4,370 $mgl^{-1}S$) at which 129 individuals were taken on one occasion, and is probably a permanent resident of ponds 4 and 5. In October 5 individuals were taken at 8,000 $mgl^{-1}Cl$ (13,000 $mgl^{-1}S$) in pond 6 but the presence of the species at this site is probably due only to transient visitors.

S.concinna appears to occupy a truly halophye position on the brinefield and this is consistent with the observations of other authors, Macan (1954) found S.concinna in Loch Gelly which has an unusually high concentration of dissolved salts and points out that of the two sites at which the species was found by Walton (1943) one was calcareous and the other was near the sea. He concludes that the species is "a rare corixid, though not a salt-marsh species &is often associated with salinity above the average

for inland waters". Savage (1971) reports that Popham found the species coastally at Colwyn Bay and Llandudno and he himself reports the abundant occurrence of the species in Watch Lane flash with salinities between 2,100 - 2,500 mgl^{-1}Cl (4,200 -4,900 mgl^{-1}S). In the Netherlands, Van der Velde and Polderman (1974) found that S.concinna made up between 11% and 61% of the corixid fauna of Klein pool where the salinity ranged from 2,500 - 3,000 mgl^{-1}S. Although not abundant on the brinefield it was taken regularly in pond 9 and on several occasions from pond 5 in salinities up to 2,600 mgl^{-1}Cl (3,590 mgl^{-1}S).

Although females of S.selecta and S.stagnalis cannot be separated, it is clear that on the brinefield, the subgenus, S.Halicorixa, finds optimum conditions in mixohaline waters. If the records of males are examined, and the assumption made that females of the two species are represented in the same ratio as males of the two species, there is an indication that the range of S.selecta extends somewhat further into high salinities than that of S.stagnalis. S.selecta alone was taken from the two most saline field ponds (ponds 7 and 8) where it was found in salinities of up to 29.6°/ooCl (49.7°/ooS), and it was also the more abundant species in the two ponds which had the next highest salinities (ponds 4 & 5). Neither species has been previously recorded in salinities greater than those of sea water in Britain and there is no evidence that S.selecta is more tolerant of salinity on the coast. Howes (1939) recorded S.selecta on its own between 21.5°/ooS and 27.9°/ooS on the Essex coast and Butler and Popham (1959) recorded both species of S.Halicorixa at 27.9°/ooS on the Spurn peninsula.

S.stagnalis occurred commonly between 800-8,100 mgl^{-1}Cl (1,000 - 10,500 mgl^{-1}S) on the brinefield and permanent populations probably existed in ponds 4, 5 and 6 where S.Halicorixa were very prolific. Savage (1971) found a slightly higher range for the species in the nearby Sandbach flashes from which S.selecta is apparently absent. S.stagnalis was common in Watch Lane Flash and Red Lane Flash in salinities of between 2,100 - 9,200 mgl^{-1}Cl

(4,200 - 16,600 $mgl^{-1}S$). Van der Velde and Polderman(1974) found S.stagnalis in association with S.concinna in Klein pool (4,600 - 5,500 $mgl^{-1}S$) where the two species composed between 70-100% of the corixid fauna,even though the dominance varied fron 8:1 with S.stagnalis predominant,to 0.5:1 with S.concinna predominant during the year. It is probable then, that competition plays some part in determining the range of S.stagnalis. Reduction of predation with increasing salinity is also probably important and may account for the abundance of S.Halicorixa in pond 4, 5 & 6 where carnivorous invertebrates were rare.

Nymphs of S.Halicorixa were taken from ponds 4, 5 and 6 between July and October 1973 (table 35) at salinities from 2,210 - 8,140 $mgl^{-1}Cl$ (3,710 - 13,700 $mgl^{-1}S$), and although the key of Cobben and Pillot (1960) does not differentiate between the two species, the nymph taken from R_1 in a salinity of 4,940 $mgl^{-1}Cl$ (8,300 $mgl^{-1}S$) was almost certainly S.selecta as this was the only species for which adults had been taken at that site.

2. Changes in the sex ratio of Sigara Halicorixa spp.

Large changes in the sex ratio of S.Halicorixa through the year were apparent (fig.25), females being only a little more abundant than males in late summer and autumn, yet three times as abundant in December and January. By comparison, the sex ratio of S.lateralis remained near to equality all year round. The pattern seen in S.Halicorixa sp is very reminiscent of that discovered in C.punctata by Walton (1943) where the female to male ratio rose from 2:1 to 7:1 in the second week in November. It reached its maximum at the beginning of December and had fallen again by January. He also found that the sex ratio of S.lateralis was constant around unity throughout the year. Walton attributed the changes in sex ratio to two factors; firstly, differential mortality, this being greater for males over winter than for females, and secondly, differential migration whereby females migrate more readily than males. Whereas Walton thought the latter factor operated during the autumn migration, Brown (1954)

found a sex ratio of virtually unity in his light trap catches which contained a total of 633 males and 632 females. Brown did however postulate that females would migrate differentially in April and May to lay their eggs. The results from the Holford ponds clarify the debate and suggest that the pattern of events governing changes in the sex ratio is probably as follows:-

Nymphs emerge between May and June when there are very few adults and the sex ratio is close to unity. They reach maturity between July and mid September and over-winter as adults. Mortality probably occurs in the adverse winter weather and is more likely to act on those individuals with least fat reserves. In biological terms, males are more dispensible than females as there is an excess of male gametes. Selection would presumably act to preserve the female gametes and to select male gametes from strong stock. (It is perhaps significant that males of S.Halicorixa are considerably smaller than the females). The sex ratio would rise in favour of the female from the time of the first winter frosts if weak males were the first to die. The ratio begins to equilibrate somewhat, early in the year, and it may be that the rate of mortality among males has declined as increasingly strong individuals remain. In 1973 there appear to have been no conditions favourable for migration before March 22nd when anticyclonic conditions lasted for three days. Conditions were then unsuitable for flight for nearly a month and the next anticyclonic weather occurred on 25th April. No large samples were taken between April and June though it appears that differential emigration of females may have contributed to a decline in the sex ratio. The pattern described stems from an assumption of strong selection for reproductive efficiency and this is less likely to occur in a species with a catholic taste for habitat than one which presumably suffers large losses during the migratory search for a very specific type of habitat

3. Colonisation of inland sites by S.Halicorixa spp.

The occurrence of coastal species at an inland site, though initially surprising, is easily credible in the light of the work

of Popham (1952, 1964) and the observations of Brown (1954) and Macan (1939). Popham (1952) recorded a flight speed of 1.28m/sec for S.distincta in still air of 20-25°C. Subsequent calculations (Popham 1964) indicated that if it was assumed that corixids began flying at about noon, distances of 50-70km could be covered in one day; and that any corixids beginning to fly at 09.30h could cover as much as 65-90km in one day,even in still air. He further adds that "once corixids have started to emigrate it would appear that they normally continue to do so until a new habitat has been found, but if they have not done so by sun-set, they normally fall to the ground and continue their flight the next day". The probability of coastal species colonising the ponds of the Holford brinefield would therefore seem to depend on the probability of corixids in flight sampling the habitat rather than being dependent on the probability of a coastal corixid being able to fly so far inland. Macan (1939) made some observations on visits of migrating corixids to a fountain at King's College Cambridge and although he recorded six local species commonly, he also took a specimen of S.selecta. He also records seeing S.lateralis in flight at an altitude of 760m in the Lake District where it is normally a rare species. The species is generally considered to migrate readily (Walton 1943, Popham 1964). Brown (1954) took S.concinna in a light trap at Rothamstead, and Southwood and Heston (1959) consider that this is also a ready migrant. The probability of the four mixohaline species, colonising the Holford brinefield thus seems to be high, especially as brine has been extracted there for over forty years. Popham (1964) believes that a temporary species will go to a more permanent habitat to lay its eggs, and in such circumstances a single egg-bearing female would be sufficient to colonise a new habitat.

4. Corixid succession in relation to salinity.

The dendrogram in figure 23 (p171) indicates distinct alterations in the corixid fauna with increasing salinity. In general terms the ponds of group A are all dominated by S.selecta and those of

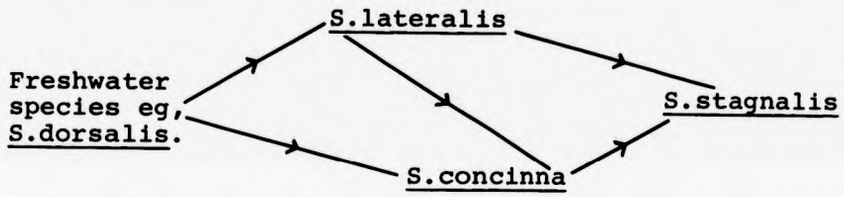
group B by S.stagnalis and S.lateralis,the former being the dominant species in ponds 4 and 5 and S.lateralis succeeding in dominance in the more dilute pond 9. Pond 6 is intermediate between groups A and B with S.stagnalis just becoming dominant over S.selecta. Group C ponds, apart from a small residue of mixohaline species, have characteristic freshwater faunas.

Although the ponds generally become progressively less saline towards the right of the dendrogram, ponds 7 and 9 are obviously anomolous. The association of pond 7 with the high salinity ponds 8 and R_1 by virtue of the presence of S.selecta was more puzzling, and the problem was not satisfactorily resolved. The pond was sampled seven times and yet only 10 individuals of corixids were taken and it thus appears that it presents an extreme environment for Corixidae for reasons other than its salinity which was very similar to that of ponds 4 and 5. The pond was very small and very shallow (circa 0.4m) and was probably susceptible to drying out, at which times sodium chloride and any other pollutants would become concentrated. The associated fauna was also characteristic of an extreme environment (table 37, p176) and contained no species with a wholly aquatic life history. Pond 9 was apparently fresher than ponds 1 and 2 and yet contained the species S.concinna and S.lateralis and small numbers of S.stagnalis which were common to the moderately saline ponds. Macan (1954) found S.concinna only rarely, but did record it in two ponds containing animal dung and also in two salt marsh ponds in close association with S.lateralis. He also found the latter species associated with ponds on sewage works and concluded that the high ionic content of the sewage was the attractive feature for S.lateralis, and in this respect the species may be considered halophye. He found that the association of Corixa punctata with this species was also very high (C=0.51). It therefore appears that the fauna of pond 9 with 19% Corixa punctata, 58% S.lateralis and 16% S.concinna is characteristic of a pond polluted with dung, and certainly the field in which pond 9 was situated was grazed by cattle and this

pond was the only one accessible to them for drinking. It is also significant to note that four of the species in pond 9 were taken only in October and that 25 of the 33 individuals of S.Halicorixa sp. were taken in that same month. The pond had not been sampled at all in the summer and these were probably late summer migrants. Five specimens of S.concinna from the same pond were taken from flight on 9th October 1973. The resident population must therefore be almost entirely composed of C.punctata, S.lateralis and S.concinna.

Considering the relationships of the ponds in terms of their corixid faunas, and also the preferred salinity ranges of the individual species on the brinefield, there is an indication that a succession of species dominance could be expected with increasing salinities and the following scheme has been constructed:-

Figure 26.



Savage confirms that his continued studies of the Sandbach flashes indicate that S.concinna may replace S.lateralis in increasing salinities (pers.comm.), and certainly in the present study the coefficient of association of S.lateralis with the other three halophye species (table 36) declines for species progressively to the right of the proposed scheme. The coefficients of association computed by Macan (1954) after an extensive survey also fit the scheme. He found an association of 0.54 between S.lateralis and S.concinna, though no association between either of these species and S.Halicorixa spp. His coefficient of association between S.stagnalis and S.selecta was

0.21. It was impossible to compute the association of these two species on the brinefield as one weakness of the test is that universal concurrence of two species makes a significant chi square unattainable. The association is, however, unmistakeable.

SUMMARY

1. Corixidae were collected from a number of field ponds of various salinities situated on the Holford brinefield.
2. Two mixohaline species, (S.stagnalis and S.selecta), and two species with low salt preferences (S.lateralis and S.concinna) were among the twelve species recorded.
3. The distribution of the major species is discussed in relation to salinity.
4. Seasonal changes in the sex ratio of S.Halicorixa are reported and interpreted in terms of movements and mortality during the life-history.
5. An analysis of the associations of the species, and the relationships of the habitats in terms of their corixid fauna, led to a schematic model of the succession of corixid species in increasingly saline habitats.

CHAPTER VII

OSMOREGULATION IN THE MIXOHALINE CORIXID *SIGARA STAGNALIS* (FIEBER).

INTRODUCTION.

The osmoregulatory ability of *Sigara stagnalis* (=*lugubris*) was investigated by Claus (1937) who measured the freezing point depression of the haemolymph of insects in a range of saline media. He concluded that the species was almost perfectly homoiosmotic in external media of up to 14°/oo salinity. At higher salinities the haemolymph was apparently regulated hypo-osmotically in external salinities of up to 20°/oo. Krogh (1939), however, cast some doubt on these results and questioned whether the specimens used had been in the external media long enough for haemolymph concentrations to have reached a steady state.

In the experiment reported in this chapter, the ability of *S.stagnalis* to regulate haemolymph sodium was investigated to gain further insight into its osmoregulatory mechanism.

The response to elevated salinities of brackish-water corixids from other parts of the world have been examined by several workers. In British Columbia Scudder et al.(1972) investigated both the total osmotic response and the ability to regulate sodium in the mixohaline species *Cenocorixa expleta* (Uhler) and found that although it is rather more tolerant of high salinities than the freshwater *C.bifida* (Hung), it still behaves essentially like a freshwater species. Knowles and Williams (1973) produced osmotic response curves for *Sigara australis* Fieber and *Agraptocorixa hirtifrons* Hale,and concluded that the ability for maintaining hypotonicity was very limited. Tones and Hammer (1975) found that *Trichocorixa verticalis interiores* Sailer from the saline lakes of Saskatchewan has an osmoregulatory mechanism similar to that demonstrated for *S.stagnalis* by Claus.

Methods.

Adult corixids were taken from pond 5 on the Holford brinefield, in January 1973 and transported to the laboratory in a 4 litre bucket in which they were temporarily stored under aeration in a cold room.

A series of sodium chloride solutions (table 38) was made up using deionised water which had then been artifically hardened to 250mgl^{-1} $CaCO_3$ (See Appendix 4). The exact strength of each solution was determined by the Mohr titration for chlorides and these concentrations are presented in table 38.

Table 38. Concentrations of sodium chloride solutions to which corixids were acclimatised.

Solution No.	1	2	3	4	5	6	7	8	9	10	11
NaCl mmol	63.98	124.0	188.0	239.5	278.0	365.0	432.0	498.0	568.0	630.0	705.3
Na mg l^{-1}	1471	2851	4323	5507	6437	8391	9932	11449	13058	14483	16215
Cl mg l^{-1}	2269	4396	6666	8492	9927	12940	15316	17656	20137	22335	25005

Ten adults of S.stagnalis were placed in 200 ml of each of these solutions in separate 100x200x80mm polythene dishes. The dishes were covered with Parafilm M and placed in a constant temperature cabinet where they were maintained at 5°C for 72h, a period which Scudder et al (1972) had found to be adequate for the acclimation of Cenocorixa sp.

Haemolymph was collected in a similar manner to that used by Scudder et al (op.cit). Corixids were removed from the sodium chloride solutions, quickly rinsed in deionised water, and then any liquid left clinging to the insect was blotted away using a Kleenex tissue. Each insect was secured by pressing its ventral surface into a smear of chilled lanolin on a microsope slide. The right forewing was removed under a binocular microscope and haemolymph collected in a 1 ml disposable glass micropipette (Microcap, Drummond Scientific Co., Broomall Pa). Haemolymph was difficult to obtain from insects which had been kept in the stronger solutions and it was sometimes necessary to remove both forewings. The contents of the micro-pipette were flushed out into 10ml of deionised water in a 20ml beaker. These samples were analysed for sodium immediately after collection, using an atomic absorption spectrophotometer (Evans Electroselenium Ltd, model 240). Three readings were taken from each 10ml sample. The experiments were repeated, especially for the intermediate solutions, to increase the number of individuals from which a mean value for haemolymph sodium was obtained. Between 15 and 69 insects were used for each point on the curve, except at the highest concentration where only 5 insects out of 114 survived.

A further 4 corixids were each introduced into 10ml of distilled water and the increase in conductivity and time of death recorded.

RESULTS

The results are presented in table 39 and are plotted graphically in figure 27 where the sodium response curve for Cenocorixa expleta (Scudder et al 1972) is superimposed.

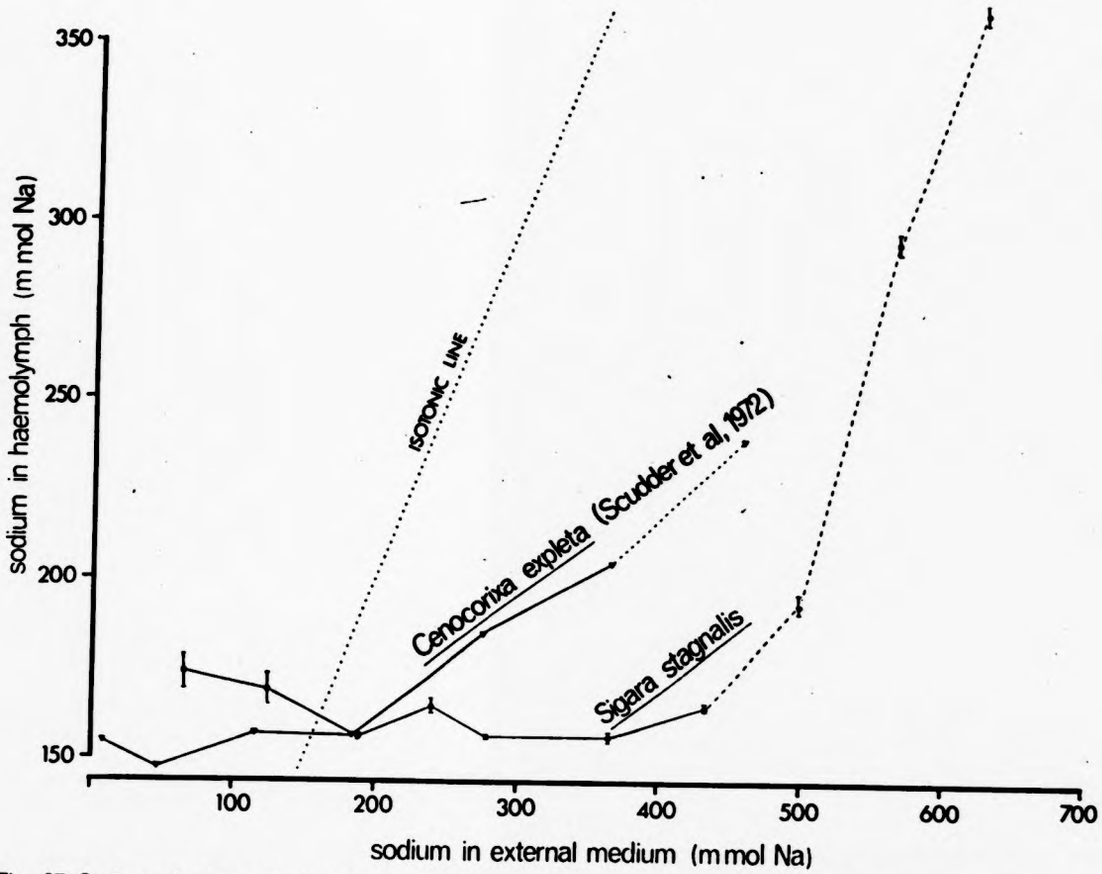


Fig. 27. Sodium balance in haemolymph of Sigara stagnalis after 72h in various concentrations of of artificially hardened sodium chloride solution, (Cenocorixa expleta compared)

Table 39. Sodium balance in haemolymph of Sigara stagnalis after 72h in various concentrations of artificially hardened sodium chloride solutions.

	Concentration of external medium									
mmol Na+	64.0	124.0	188.0	239.5	278.0	365.0	432.0	498.0	568.0	630.0
(°/ooS)	3.99	7.53	11.24	14.25	16.5	21.55	25.5	29.36	33.45	37.07
Na^+ in haemolymph (x) (mmol)	176.2	169.6	156.6	165.3	156.6	156.6	165.3	193.6	295.8	364.1
standard deviation of x (mmol)	17.4	17.2	5.6	8.7	17.4	8.7	5.6	10.4	13.0	8.7
No. insects used	18	15	37	35	46	69	47	27	45	5

Although mortalities occurred in all acclimation dishes, presumably as natural mortality is high at this time of the year, they only departed significantly from the mean (14.3%) in solutions 9, 10 and 11 where mortality reached 37%, 32% and 96% respectively.

The sodium response curve shows that Sigara stagnalis can perform hypertonic regulation in external media of between 0 & 162 mmol Na^+, and then hypotonic regulation up to salinities of around 430mmol Na^+. At higher salinities, the sodium content of the haemolymph begins to increase and increasing mortalities presumably signify a breakdown in regulation.

The four corixids introduced to distilled water at a temperature of 10°C at time 0h, died at 24h, 49h, 75h and 76.5h, at which time external medium had an increased conductivity of between 4.5 μmhos cm^{-1} and 11.0 μmhos cm^{-1} (figure 28). Initial rate of increase of conductivity was around 0.56 - 0.61 μmhos cm^{-1} h^{-1} at 10°C.

DISCUSSION

The sodium response curve for S.stagnalis apparently confirms that it can regulate haemolymph sodium in hyperosmotic media with concentrations up to 430 mmol NaCl (equivalent to 25.4 °/ooS). This value is greater than that indicated by the total osmotic response curve produced by Claus (1937), but whereas his work was conducted at room temperature, corixids used in the present study were acclimated at 5°C, and this would account for the discrepancy. Scudder (1969) demonstrated that a temperature difference of this order could lower the salinity survival limit of Cenocorixa expleta by as much as a third, and Tones and Hammer (1975) demonstrated a similar relationship between temperature and salinity tolerance limit for Trichocorixa verticalis interiores. Although Claus recorded mortalities in external media of 20°/ooS, he found that the body fluid remained hypotonic at salinities far above this. Unfortunately the limitations of his method made it impossible for him to assay these. Nevertheless, this observation coupled with

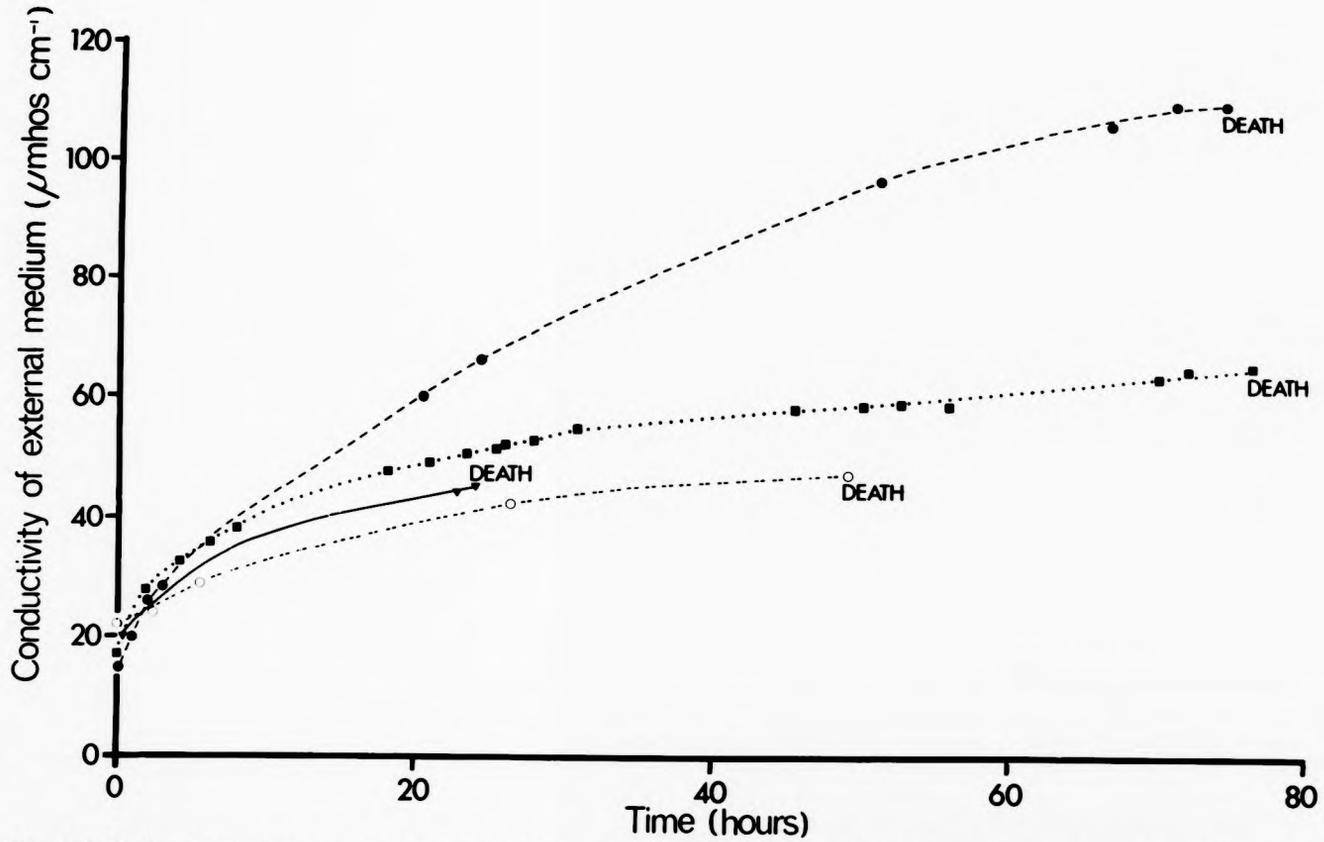


Fig. 28. Salts lost to external medium by four individuals of Sigara stagnalis placed in distilled water.

the results of the current experiment indicate that S.stagnalis may have powers of maintaining hypotonicity of the same order as those demonstrated for T.verticalis interiores (Tones and Hammer 1975), and indeed natural breeding populations of S.stagnalis have been recorded from salinities as high as 20.75°/ooS (Lansbury 1954).

The concentration at which regulation began to break down at temperatures of 5°C in the present study (430mmol NaCl) is equivalent to a freezing point depression of 1.44°C (using the tables of Schlieper, in Remane & Schlieper (1958), p251), and the median tolerance limit of S.stagnalis was between this value and a freezing point depression of 2.02°C (630 mmol NaCl). This compares well with the median tolerance limit for T.verticalis interiores exposed to external media with a freezing point depression of 1.64°C and acclimated at 13°C (Tones and Hammer, op.cit). It is apparent then that both species , albeit in different hemispheres, have developed similar mechanisms for osmoregulation, though S.stagnalis additionally maintains a rather higher haemolymph concentration than T.verticalis interiores.

The ability to regulate the sodium ion seems to be a fundamental requirement for corixids inhabiting mixohaline and brackish water. In Cenocorixa expleta sodium is regulated, even though total osmotic pressure of the haemolymph is allowed to increase almost isotonically with the medium. The point at which the sodium regulation breaks down is also the point at which the haemolymph becomes hypotonic and at which mortalities first occur (Scudder et al, 1972). The osmotic response in Sigara australis and Agraptocorixa hirtifrons is similar (Knowles and Williams, 1973), and in all three corixids the mechanism is characteristic of an animal which, despite its permeability to water and salts, can regulate the electrolyte fraction of the blood, allowing only the non-electrolyte fraction to increase in media of increasing osmotic pressure. The current experiment shows that S.stagnalis can regulate sodium very powerfully, but as total osmotic pressure of the haemolymph is also regulated, an additional osmoregulatory mechanism must be post-

ulated. Osmoregulation in T.verticalis interiores shows the same characteristics and it can be postulated that regulation of total osmotic pressure must be achieved either by a reduced permeability or by elaboration of hypertonic urine.

A reduction in permeability seems unlikely; in the present study rate of salt loss appeared rapid (0.56 - 0.61 μmhos $cm^{-1}h^{-1}$ at 10°C) and no individual survived the distilled water for longer than 76.5h. This corresponds well with periods of acclimation to the medium required by C.expleta and C.bifida (Scudder et al 1972) and for T.verticalis interiores (Tones and Hammer 1975). Furthermore Holgate (1956) and Staddon (1963, 1964, 1966) have both demonstrated that the cuticular permeability of aquatic Hemiptera is much higher than in terrestial insects, and as respiration in corixids relies on cuticular permeability to atmospheric oxygen, low permeability would seem disadvantageous. Further research is necessary to resolve whether S.stagnalis and T.verticalis interiores have the ability to elaborate hypertonic urine.

SUMMARY.

1. Levels of sodium in the haemolymph of Sigara stagnalis in various concentrations of artifically hardened sodium chloride solutions were investigated.

2. The sodium content of the haemolymph was regulated in external media containing at least 365 mmol NaCl which is much higher than the concentrations to which Cenocorixa expleta can regulate sodium. The latter behaves in essentially the same manner as a freshwater form,whereas S.stagnalis appears to be a true mixohaline form.

3. Tolerance of elevated salinity was in accord with field records and was similar to that found by Claus (1937) for S.stagnalis and that found for Trichocorixa verticalis interiores by Tones and Hammer (1975). Both corixids seem to possess a similar mechanism for osmoregulation in high salinities.

4. The possibility of decreased permeability is discounted and the ability to produce hyper-osmotic urine is suggested as the possible mechanism by which the osmotic pressure of the blood is maintained hypotonic to the medium.

CHAPTER VIII

SALINITY TOLERANCE THRESHOLDS OF THREE FRESHWATER INVERTEBRATES.

STATISTICAL TREATMENT OF THE ACUTE RESPONSE TO LETHAL SOLUTIONS.

As assay in studies of tolerance requires a quantal response, and the most easily definable response is death, an acute test was used to assess the severity of sodium chloride solutions for some freshwater animals.

Mortality in a lethal solution exhibits a log-normal distribution, and if cumulative percentage mortality is plotted against dose, a skewed sigmoid results. Solution of dose - percent effect curves of this type requires linear transformation which is achieved by the conversion of doses to logarithms and of cumulative percentage mortalities to probits, (e.g. fig.29). A regression line can then be fitted to each curve and these solved for Median Effective Dose (ED_{50}) with confidence limits for given exposure times. The method of Finney (1952) has been widely used as it makes allowance for control mortality, and as the Keele (Elliott 4130) computer was programmed for his method of probit analysis, it was used for the solution of dose-effect data in this study. Data are however presented graphically in figures 29 and 30.

EXPERIMENTAL METHODS.

Baetis rhodani, Simulium ornatum and Gammarus pulex were all used as experimental animals and were obtained from the Keele stream and held under aeration in a cold room prior to the experiments. A range of solutions of sodium chloride was made up in water artificially hardened to 250 mg $l^{-1} CaCO_3$ (Appendix 4) and stored in a constant temperature cabinet at $10^{o}C$. Sterile sectioned petri dishes, 100mm x 100mm x 20mm, each of which had 25 compartments $(20mm)^3$ (Griffin and George) were prepared to contain the experimental animals during trials. A heated rod was used to make holes

(diameter 1mm) between compartments to allow free exchange of the test solution. 125ml of experimental solution was poured into each petri dish and experimental animals allocated to individual compartments at time Oh. Lids were placed over the petri dishes which were kept under illumination in a constant temperature cabinet at 10°C. Dishes were inspected at time intervals which were generally logarithmic, and number of deaths recorded. Where neither movement nor pulse were detectable, the response of animals to a blunt probe was tested. Total failure to respond was taken to indicate death and the individual was removed from the dish. The water was changed every 48h by removing the lids from the dishes and placing a fine mesh sheet over the top. Each dish was carefully tilted to an angle of 90° so that the solution drained through the mesh whilst the experimental animals were retained in their original compartments. 125ml of the appropriate fresh stock solution was then poured into each dish. The range of solutions, number of specimens and the inspection programme for each experiment are presented in table 40 for each experiment. Experimental design was generally improved in successive experiments by alterations to the range of test solutions, increases in numbers of test organisms, and extension of the inspection programme. Animals were not fed during the experiments.

RESULTS.

The results are summarised in table 41, the dose-effect curves for experiment 6 are presented in figure 29 and the toxicity curve is presented in figure 30.

DISCUSSION.

All three species showed a vulnerability to increased sodium chloride concentrations that is typical of limnobiont species incapable of regulation in hypertonic media. The 24 hour LC_{50} for all species on all trials is given in terms of equivalent total salinity in table 42, and fell between 8°/ooS and 10.5°/ooS for each species.

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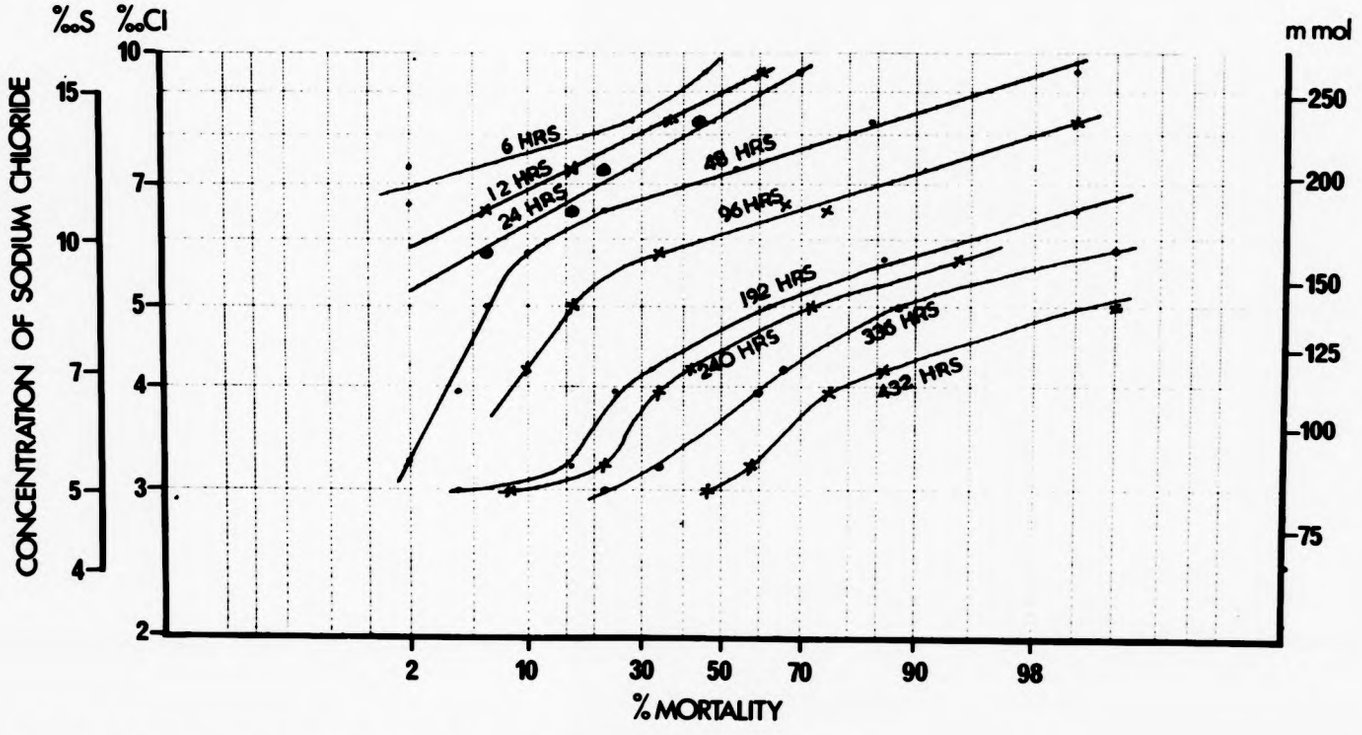

FIG. 29. DOSE EFFECTS OF SODIUM CHLORIDE ON GAMMARUS PULEX.

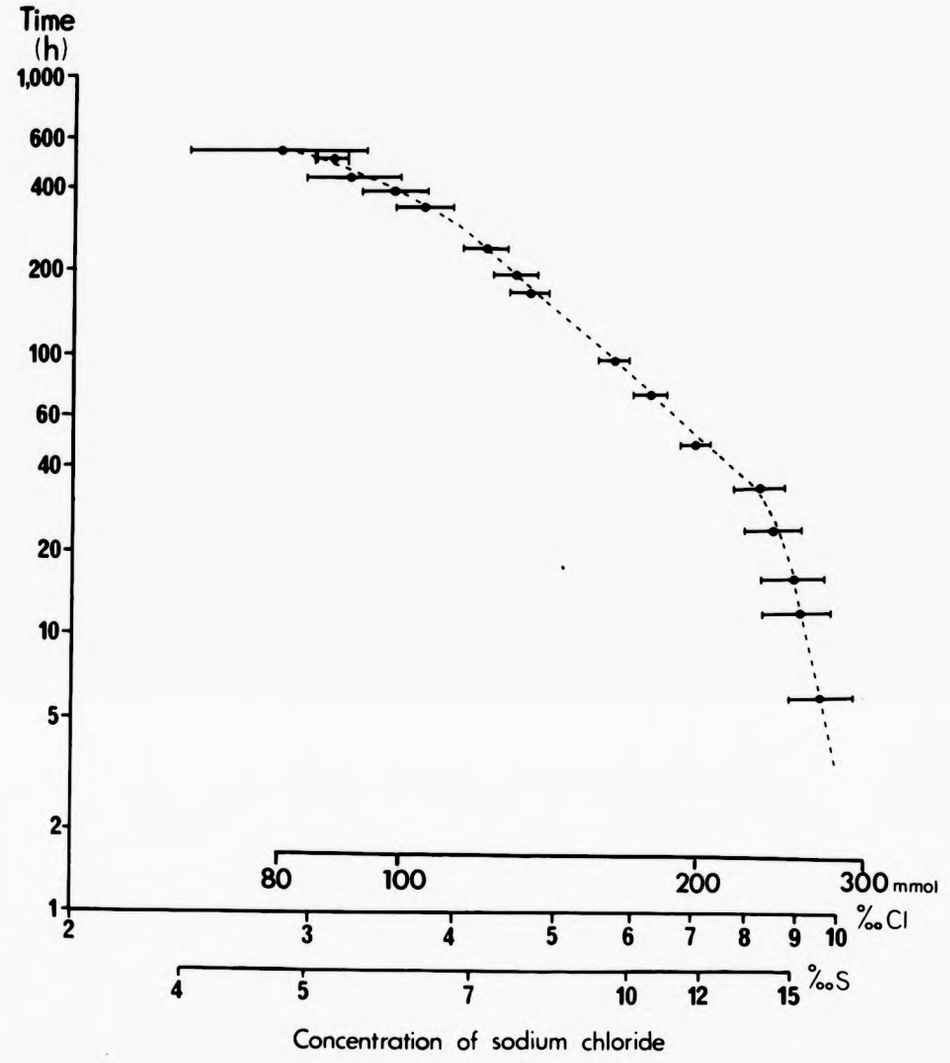

Fig. 30. Lethal effects of sodium chloride on Gammarus pulex.

Table 40. Experimental design and solutions used to assay the tolerance of various invertebrates to sodium chloride in solution.

Expt No.	Species	Duration of expt. (h)	Number individuals in each expt.	Dose (mmol NaCl)
1	Baëtis rhodani	0 - 52	25	Control
				124.1
				141.0
				160.7
				183.3
				205.9
				234.1
				267.9
2	Simulium ornatum	0 - 96	25	Control
				76.1
				93.1
				110.0
				132.5
				157.9
				191.8
				231.2
3	Gammarus pulex	0 - 241	10	Control
				100
				120
				140
				160
				180
				200
4	Gammarus pulex	0 - 96	50	Control
				76.1
				93.1
				110.0
				132.5
				157.9
				191.8
				231.2

Table 40 continued.

Expt No.	Species	Duration of expt. (h)	Number individuals in each expt.	Dose (mmol NaCl)
5	*Gammarus pulex*	0 - 552	50	Control
				84.6
				95.9
				110.0
				124.1
				141.0
				160.7
				183.3
				205.9
				234.1
				267.9
6	*Gammarus pulex*	0 - 98	50	Control
				112.0
				133.0
				158.5
				191.5
				231.0
				280.5

Table 41. Tolerance of various invertebrates to sodium chloride in solution.

Expt. No.	Species	Time (h)	Median lethal dose (mmol NaCl)	Fiducial limits of LD_{50} (mmol NaCl)
1	Baëtis rhodani	24	254.8	222.1 - 282.3
		48	175.2	164.1 - 187.1
2	Simulium ornatum	24	259.1	171.8 - 391.1
		48	205.1	184.9 - 236.1
		96	118.4	108.9 - 128.8
3	Gammarus pulex	24	231.8	145.2 - 370.1
		48	187.9	151.2 - 233.6
		80	165.8	151.6 - 181.2
		156	119.5	105.2 - 135.7
		241	116.0	98.9 - 136.0
4	Gammarus pulex	24	289.9	171.6 - 489.8
		48	226.3	210.1 - 242.8
		96	164.7	156.0 - 173.9
		192	132.5	124.4 - 141.1
5	Gammarus pulex	6	268.6	249.9 - 293.3
		12	254.9	234.7 - 277.0
		16	252.1	233.2 - 272.6
		24	239.8	223.7 - 256.9
		34	231.4	218.0 - 245.6
		48	197.7	189.6 - 206.1
		72	176.4	169.3 - 183.6
		96	162.0	168.6 - 155.6
		168	132.1	126.2 - 138.2
		192	128.1	122.2 - 134.3
		240	120.2	114.5 - 126.1
		336	105.1	99.0 - 111.5
		384	99.2	93.2 - 105.5
		432	90.7	82.5 - 99.7
		480	87.9	86.0 - 89.8
		552	79.6	67.6 - 93.8
6	Gammarus pulex	24	247.9	225.9 - 272.0
		34	236.4	217.2 - 257.3
		48	226.6	209.8 - 244.3
		63.5	220.8	209.9 - 232.3
		98	201.6	191.9 - 211.7

Table 42. The median lethal concentrations of sodium chloride for three invertebrate species (expressed as total salinity).

	24 hour exposure.	48 hour exposure.	96 hour exposure.
Gammarus pulex.	8.1°/ooS-10.5°/ooS	6.9°/ooS-8.3°/ooS	6.1°/ooS-7.1°/ooS
Simulium ornatum	9.4°/ooS	7.5°/ooS	4.5°/ooS
Baëtis rhodani	9.3°/ooS	6.5°/ooS	-

The incipient LC_{50} (lethal concentration for 50% of individuals on long exposure) is, however, the most useful criterion of toxicity and as a general rule the 96 hour LC_{50} is a good substitute for this (Sprague, 1969). For S.ornatum this value was 4.5°/ooS and for G.pulex it was between 6°/ooS and 7°/ooS. These values are consistent with the observed range of the species in their natural habitat. The limit of the range of S.ornatum in Crow Brook was between mean salinities of 3.6°/ooS and 3.7°/ooS and as these are not much lower than the 96 hour LC_{50}, it appears that the range of this species in that situation is governed solely by physiological factors. For G.pulex, however, the limit of its range in Crow Book was 2.6-2.7°/ooS, which is considerably lower than the 96 hour LC_{50}. The range of G.pulex in the stream must therefore be restricted by factors which become operative before salinity levels become lethal. If the assumption is made that the organism is under stress, even at sub-lethal salinities, perhaps due to the increased energy output required for maintaining the ionic balance of the haemolymph, then

it follows that ecological factors may become limiting. The distribution of Asellus aquaticus for example, suggests that it is a successful competitor in the range 2.6°/ooS to 3.6°/ooS. Elsewhere G.pulex was found in salinities of up to 5.2°/ooS and this is of the order of natural tolerance consistent with a 96 hour LC_{50} of 6-7°/ooS.

Although a 96 hour LC_{50} was not obtained for B.rhodani, the 48 hour LC_{50} indicates that it is probably more sensitive than the other two species. The 24 hour LC_{50} was, however, almost indentical to those for G.pulex and S.ornatum and it seems probable that the greater sensitivity on long exposures is the result of a higher permeability to water and ions. The limit of the range of B.rhodani in Crow Brook was lower than that for S.ornatum but the same as that for G.pulex (2.6-2.7°/ooS), and in this case the range may well be limited physiologically rather than ecologically.

Values for the toxicity thresholds for North American stream invertebrates exposed to brine wastes have been produced by Clemens and Jones (1954). The 96 hour LC_{50} of brine wastes for Baëtidae was 5.3°/ooS, for Hexagenia sp. (Ephemeroptera) it was 7.5°/ooS, and for Hyalella azteca (Amphipoda) it was 5.6°/ooS. These values are not inconsistent with the tolerances produced in this investigation for the equivalent British species.

Although the chief criteria for toxicity in this series of investigations were 24 hour and 96 hour median thresholds, sufficient data were available to draw a toxicity curve for G.pulex(fig.30).Median survival times were plotted against sodium chloride concentrations on logarithmic paper and the resultant curve revealed an unusual pattern of toxicity. Curves plotted in this way normally resemble a rectangular hyperbola, becoming asymptotic to the time axis at the point of lethal threshold concentration, and asymptotic with the concentration axis at the minimum time for lethal action. The

toxicity curve produced for G.pulex in sodium chloride solutions did not take this form, and while the asymptote with the concentration axis was only absent because the appropriate doses were not investigated, the failure of the curve to steepen towards the time axis is atypical. Although the curve is climbing steeply between 24-34h and is not far from being asymptotic, there is a sudden change in rate of mortality after about 34 hours exposure. There was, however, no mortality in the control at this stage. Live young were produced in the control on the eighth day and the first death did not occur until the tenth day.

It appears then that after 34 hours, there is some synergistic effect acting with the sodium chloride, which reduces the tolerance of the shrimps for sodium chloride. While the curve is probably representative of the direct lethal effects of sodium chloride in the early part of the experiment, beyond 34 hours it must also represent the effect of an additional factor on salinity tolerance. Furthermore, this factor only operates under the experimental conditions. Not only does G.pulex survive readily at these salinities in the wild, but its normal haemolymph concentration is 135 mmol NaCl (Sutcliffe 1967b), well above many of the concentrations that were causing mortality in the experiment. Several explanations present themselves:-

(a) The experimental animals were starved during trials and they may have been unable to sustain ionic regulation with a net energy loss. This does however imply that osmotic regulation is more demanding in isotonic media than in dilute media.

(b) Ionic imbalance in the medium may effect the ability to osmoregulate, and it is possible that anomolous ionic proportions occurred, e.g. in the calcium : sodium ratio or the potassium : sodium ratio, which did not occur in the natural habitats that were investigated. Schlieper (in Remane and Schlieper, 1958, p.310) sums up these effects succinctly; "Such limiting effects of ions are to be expected in every case when an animal species is no longer

capable to compensate - by means of its regulatory mechanisms - for a deficit or a surplus of any ion, and thus no longer able to maintain an adequate ionic balance in its cells".

In this case calcium may be important as not only does G.pulex moult frequently, but calcium is also an important co-enzyme in sodium transport.

(c) Physical damage may be caused in the small cells in which the experimental animals were confined. Damage to the integuement would render the shrimps more vulnerable to osmotic stress.

Even though the explanation for the phenomenon remains elusive, it does illustrate the desirability of plotting complete toxicity curves rather than relying on median lethal concentrations for specific exposure times. It is therefore important to note that the lethal threshold concentration is certainly less than 231.4 mmol NaCl ($8.5^{o}/_{oo}S$) and that whilst the 24 hour LC_{50} is probably reliable, the 48 hour and 96 hour LC_{50} for G.pulex are probably rather low.

SUMMARY

(1) Toxicity data have been produced for three species of stream invertebrates in sodium chloride solutions.

(2) The median lethal concentrations for 24 hour, 48 hour and 96 hour exposure periods were consistent with the range of tolerance recorded in the field for Simulium ornatum and Baëtis rhodani. The natural range of Gammarus pulex was less extensive than the tolerance data would indicate and this is attributed to ecological factors.

(3) The toxicity curve for G.pulex was anomolous and explanations for the high mortality rates in low experimental concentrations have been suggested.

CHAPTER IX

DISCUSSION

INTRODUCTION

The effects of salinity on the various communities investigated in this study indicate that there are a number of ecological changes that are characteristic of inland saline waters. Primarily, as salinity increases there are orderly changes in the composition of the faunas, these being manifested in the sequential disappearance of various categories of limnobiont organisms and the appearance of salinity tolerant and true brackish water forms. Indicator species and groups can be identified for various parts of the sequence. Secondly, increases in salinity are characterised by a gradual decline in the total number of species. Despite the rapid disappearance of freshwater species some diversity is maintained into relatively high salinities by an increase in the number of halophye species. At high salinities (eg. above $15^{o}/_{oo}S$), the fauna is very impoverished. The third characteristic that can be identified is a systematic change in the total number of individuals. The number of organisms per unit area initially decreases with increasing salinity, but as competitors are removed the salinity tolerant species proliferate in numbers and this results in an overall increase in population density. An analysis of these fundamental ecological changes has led to the derivation of a method for the biological assay of inland saline waters, (table 8, p 66), and has made a contribution towards producing a system for their classification. These changes and their application to biological monitoring and classification will now be discussed.

Changes in the composition of the fauna.

A continuous sequence of changes in the composition of the fauna was most clearly seen in Crow Brook where those components which disappeard first with increasing salinity were designated as stenohaline and euryhaline species of the first degree. The next component to disappear as salinity increased was designated as euryhaline to the second degree, and the limnobiont forms which were most tolerant of the salinity were designated as

euryhaline to the third degree. As some species, particularly those in the last category, proved to be more prolific in moderate salinities than in low salinities (c.3-6^{o}/ooS), these were termed halophye. The remaining species found in high salinity waters fell into two categories, firstly those which were genuine brackish water species, and secondly those which were tolerant across the whole spectrum of salinity and which were designated as holeuryhaline.

The same categories, with approximately the same salinity limits proved applicable to the data from the Trent and Mersey Canal, though it became apparent that some halophye species were more tolerant than others and it was felt necessary to split this component into halophye organisms of the first degree and halophye organisms of the second degree. The subdivision proved particularly appropriate when the faunas of the more saline brine pits and springs were analysed with reference to salinity and it became apparent that many of the Diptera were most prolific in salinities of around 6-16^{o}/ooS.

The succession of faunal components with increasing salinity as deduced from the series of investigations reported in earlier chapters is presented schematically in figure 31 which is based on an analysis of numbers of species comprising each faunal component at the various salinities. Although assignment of species to the various categories was somewhat subjective in the first instance, it was considered that as the classification had proved applicable to all sites, it had some objective validity and could therefore be used predictively. Change in faunal composition was therefore one of the parameters used to construct a matrix of halobic indices (table 8, p66) which were appropriate for designating the biological severity of a saline environment.

As detailed biological analysis is not always possible, the task of assaying the effects of salinity can be greatly simplified if indicator groups can be isolated, and in the present series of

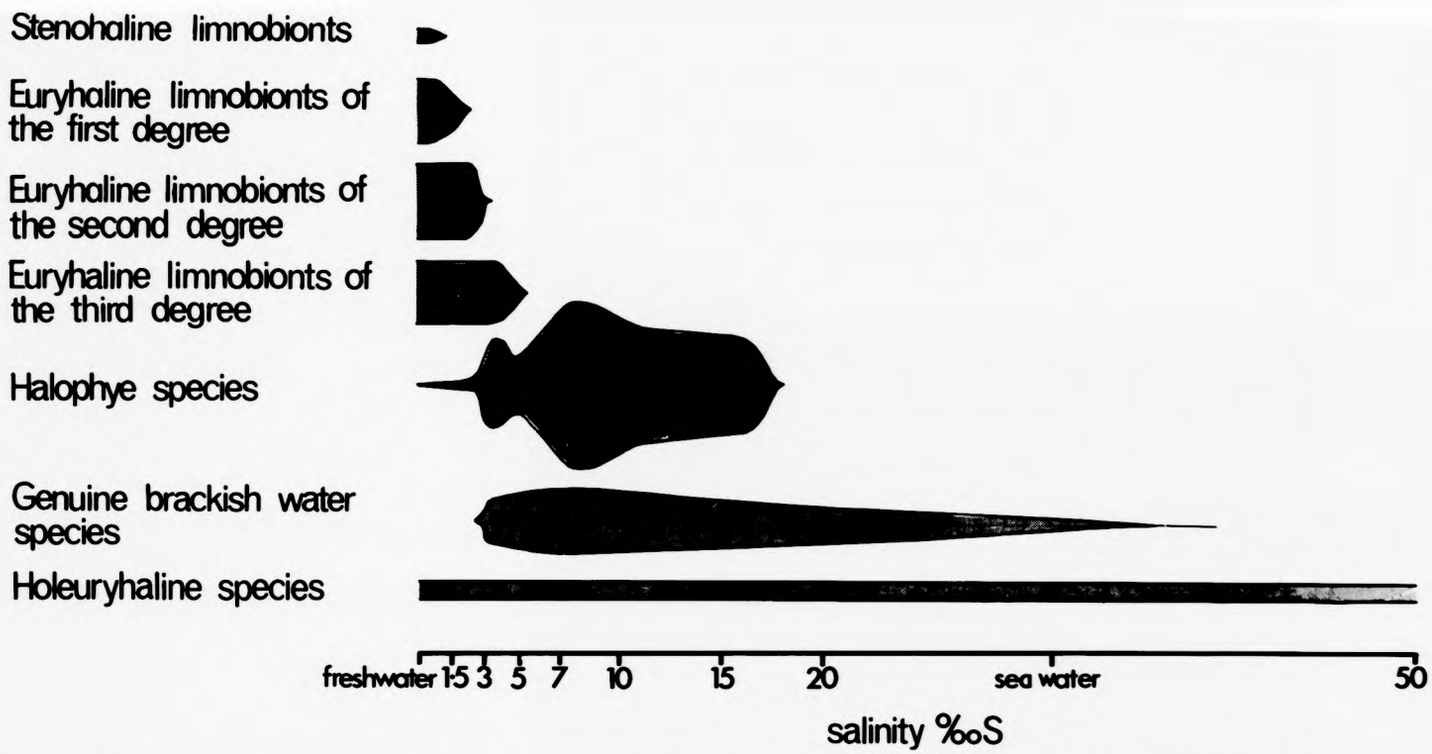

Fig. 31. Schematic representation of the abundance of various components of the fauna with increasing salinity.

investigations it has been possible to identify certain species and groups which are characteristic of the major faunal categories. Previous records of many of these groups have been discussed in earlier chapters and their ranges showed a high level of agreement with the ranges of salinity tolerance found in the present study.

Apparently there were very few species which were stenohaline (0-0.5°/ooS), though as none of the sites that were investigated had salinities close to 0.5°/ooS there was little opportunity to separate numbers of this category from those species which were euryhaline to the first degree. Hydropsyche pellucidula is however almost certainly stenohaline, not having been found at salinities greater than those at station 2 of Crowbrook.

Euryhaline species of the first degree (0-c.3°/ooS) included all the leeches found, all of the molluscs except Potamopyrgus jenkinsi, and also the triclad Polycelis tenuis. All the Plecoptera and Ephemeroptera that were recorded fell into this category, and all the Trichoptera except Hydroptila tineoides and Limnephilus affinis. Some of the Chironominae must also be regarded as euryhaline 1°, for example Potthastia longimanus, Synorthocladius semivirens and Pentaneura sp.

Species that were euryhaline to the second degree were generally recorded from freshwater to salinities of around 4.5°/ooS, though the range would obviously be restricted in poikilohaline environments (to 3.7°/ooS in Crow Brook). The most useful indicator species for this category, by virtue of its wide distribution in Britain is Gammarus pulex, and although Eiseniella tetraedra and Dendrocoelum lacteum are less common, they too are representative of the category. The chironomids Brillia modesta and Eukiefferiella claripennis and the empid Hemerodroma sp. are also euryhaline 2°.

The range of salinities from which euryhaline species of the third degree were found was from freshwater to around 7°/ooS, though a reduction of the upper limit by 15-20% was again seen in the poikilohaline environment of Crow Brook (to around 5.5°/ooS). All of

the tubificids that were identified fell into this category (Tubifex tubifex, Limnodrilus cervix, L.claparedeanus, L.hoffmeisteri) although brackish water members of the family are known (Brinkhurst 1971), Lumbriculus variegatus was also euryhaline 3°. The category also includes Simulium ornatum, many of the more tolerant chironomids (Rheocricotopus effusus, Diplocladius cultriger, Euorthocladius thienemanni, Eukiefferiella sp., Cryptochironomus sp, Glyptotendipes sp) and probably most of the Coleoptera.

Many of the species that are halophye to the first degree (c3°/oo-6°/ooS) are essentially euryhaline to the second and third degree and share the same upper tolerance limits. The distinction is that the halophye species reach their maximum abundance in moderate salinities, probably as a result of the absence of competitors or predators. Perhaps the clearest example of this was the proliferation in numbers of Asellus aquaticus at salinities where Gammarus pulex had been eliminated from Crow Brook. Apart from having a peak of abundance in moderate salinities, A.aquaticus is essentially euryhaline 3° in its upper tolerance limit. Although the tetraploid British form of Potamopyrgus jenkinsi has apparently become intolerant of high salinities, it still retains a rather greater tolerance than the majority of molluscs and can utilise this advantage to occupy a halophye position as seen in Crow Brook and probably also in the Trent and Mersey Canal. Hydroptila tineoides responded in the same way in Crow Brook possibly taking advantage of the elimination of Hydropsyche spp. The muscid, Limnophora sp. and many of the rheophilic chironomids were also halophye 1° (Macropelopia sp., Apsectrotanypus trifascipennis, Psectrotanypus varia, Microtendipes sp., Prodiamesa olivacea, Metriocnemus atratulus).

Members of the other halophye component of the fauna were most abundant in salinities between 6°/ooS and 16°/ooS and these included forms that are strongly euryhaline by virtue of increased impermeability or by possession of some special mechanism whereby salt balance can be maintained in high salinities. Most of the species

which are to some degree impermeable, but unable to perform hypo-osmotic regulation belong to the Diptera. Chironomus riparius, Procladius sagittalis and Cricotopus sylvestris all tolerate salinities up to c.16°/ooS and presumably achieve this by a reduction of permeability and possibly also by a reduction in the size of the anal papillae. Impermeability is more complete in those Diptera associated with dung (Dolichopodidae, Psychodidae, Scatophagidae and Sepsidae),and in the Ceratopogonidae,Tabanidae and Syrphidae,all of which must be considered Halophye 2°. Culex pipiens and Limnephilus affinis are also halophye at high salinities. Both show hypertonic regulation and their tolerance of elevated salinities is enhanced by a high organic component in the haemolymph which contributes to its total osmotic pressure (Bayly,1972; Sutcliffe 1959). L.affinis is also capable of a slight degree of hypo-osmotic regulation (Sutcliffe, op.cit).

Genuine brackish water species were taken as those species which are not normally associated with unpolluted freshwaters and which breed in brackish waters. These included the estuarine amphipods Gammarus duebeni, G.tigrinus and G.zaddachi, the mixohaline corixids Sigara stagnalis and S.selecta, and the halobiont Diptera Chironomus aprilinus, C.salinarius, Halocladius varians and Aedes detritus. Some of these species are better indicators than others and whereas the presence of G.duebeni only indicates that salinity exceeds 0.5°/ooS, the presence of G.zaddachi and G.tigrinus gives a more sensitive indication of the degree of salinity. The former was frequent only where salinities were greater than 4.5°/ooS and the latter was most successful where the salinity was no greater than 5°/ooS. The two mixohaline corixids are also good indicators of salinity and their distributions on the Holford brinefield suggest that where there is a breeding population, salinity is probably between 3.7°/ooS and 14°/ooS, though the observations of Lansbury (1954) suggest that the upper limit may be as high as 21°/ooS. Of the genuine brackish-water species, only G.duebeni and A.detritus are commonly found in salinities exceeding that of sea water.

The holeuryhaline component of the fauna was mainly characterised by the very impermeable Diptera (Nemotelus notatus, Leptocera sp, Atissa pygmaea, Ephydra riparia), though the oligochaetes Nais elinguis and Lumbricillus reynoldsoni may also belong to this category. By definition these species can be present in waters of all salinities and therefore are not good indicators. E.riparia does however show a strong halophye tendency in salinities exceeding 15°/oo at which most of the other Diptera have been eliminated (eg. Aldersey Spore and Lower Wych brine pit).

Although each of the major faunal groups contains characteristic species, many of which are good indicators of certain salinity ranges perhaps the most useful taxon for salinity assessment across a broad range of salinities is the Corixidae. As corixids are ready migrants it is unlikely that a species would be found in any abundance in unsuitable habitats. If it could be assumed that the ponds of the Holford brinefield are typical of inland saline waters, deductions could be made about the salinity ranges within which certain combinations of corixids occur (see figure 24, p172). One prerequisite for predictions of salinity would be the presence of either Sigara stagnalis or S.selecta as these are the only species exclusively associated with saline water(Southwood & Leston 1954a;Macan 1954), though the presence of S.concinna probably indicates that conductivity is above average for freshwaters. Where freshwater corixids occur in abundant numbers with occasional specimens of the two mixohaline species, salinity would probably be below 0.3°/ooS, but where the only freshwater corixid accompanying them was S.dorsalis, salinity would probably be between 0.3°/ooS and 2.0°/ooS. Where the halophye corixids, S.concinna and S.lateralis were the only other corixids found with S.stagnalis and S.selecta it would be probable that salinity was between 2°/ooS and 5.0°/ooS. Salinities at which only the two mixohaline species occurred would probably be between 5.0°/ooS and 15°/oo and where S.selecta occurred alone it would be likely that the salinity fell between 15°/ooS and 28°/ooS. While such a scheme could in no way be treated as

absolute (for example Lansbury, 1954, found S.stagnalis abundantly in 21°/ooS), and while the salinity boundaries need confirmation, the scheme does provide good general guidelines for the assessment of salinity and could be made more objective with further research.

Changes in the total number of species.

Figure 31 gives a schematic representation of how the number of species contributing to each major component of the fauna changes with increasing salinity. The number of limnobiont species, (and the halophye 1° species must be included with these), shows an overall decline with increasing salinity with diversity reaching a minimum around 5-6°/ooS. The halophye 2° component and the brackish water component which are in some way equipped to either tolerate high salinities or regulate their salt balance, reach maximum diversity in the absence of the freshwater component at around 7°/ooS. Beyond these salinities the number of species declines, and in particular, many of the Diptera are eliminated around 16°/ooS. This pattern is in agreement with the generalised scheme produced by Zenkevitch (1959) for the inland seas of the U.S.S.R.

The scheme presented here is derived by an analysis of the faunas of the saline habitats investigated in this study and therefore has an empirical basis. In Crow Brook, for example, (see figure 10, p 48) the total number of species began to decline where mean salinities were between 0.2°/ooS and 3.4°/ooS (stations 2 and 3), and declined quite rapidly where mean salinities were between 5.3°/oo and 6°/oo (stations 7 and 8).

Although 14 species remained at these salinities, they included the brackish-water species Gammarus duebeni and 7-8 species of the resistant Diptera (table 6, p 44). Individuals of limnobiont species were scarce.

The fauna of the Trent and Mersey Canal also showed a decline in diversity with increasing salinity (figure 18, p 99) though the profile was somewhat confused by differences in sampling intensity,

presence of other pollutants and, probably, historic salinity conditions. It was nevertheless apparent that a significant decline in number of species occurred between 0.1‰ & 3.4‰S, (ie, between stations 1-5 of the freshwater zone and station 15 of the reduced salinity zone), and the remaining stations had 7 or less non-dipterous limnobiont species (table 17, p89). At mean salinities of over 5.7°/ooS only dipterous and genuine brackish water species remained.

There is some indication then, that a salinity of around 6°/ooS represents the upper tolerance limit for limnobiont species, and confirmation for this observation comes from the data collated from brackish waters by Remane (1934) and which is presented in figure 32. It appears that the number of limnobiont species in brackish waters of increasing salinity follows the same pattern as that seen in Crow Brook and the Trent and Mersey Canal. There is a rapid fall in diversity at salinities above 0.5°/ooS, and the minimum number of species is at about 6°/ooS. It was on the basis of changes in faunal diversity such as those demonstrated by Remane, that the Symposium on the Classification of Brackish Waters (Anon, 1958) took 0.5°/oo as the boundary between the Limnetic and Oligohaline zones, and 15°/oo as the boundary between the Oligohaline and Mesohaline zones. In the light of the data collated in the current series of investigations, these boundaries would also appear appropriate for chloride based inland saline waters.

The similarity between the faunas of marine-brackish waters and those of inland saline waters ceases beyond the upper oligohalobic boundary, however. Inland saline waters with salinities greater than 6°/oo were dominated by the Diptera whereas in brackish waters these are absent and the fauna comprises only marine and genuine brackish water species (Hedgpeth 1951, Beadle 1959, Bayly 1968). The Shrewbridge pool, Aldersey Spore and Aldersey station 1 all had a large number of species and it was the Diptera which accounted for the majority of these (table 31, p149). Aldersey Spore had less species than Aldersey station 1 and this was

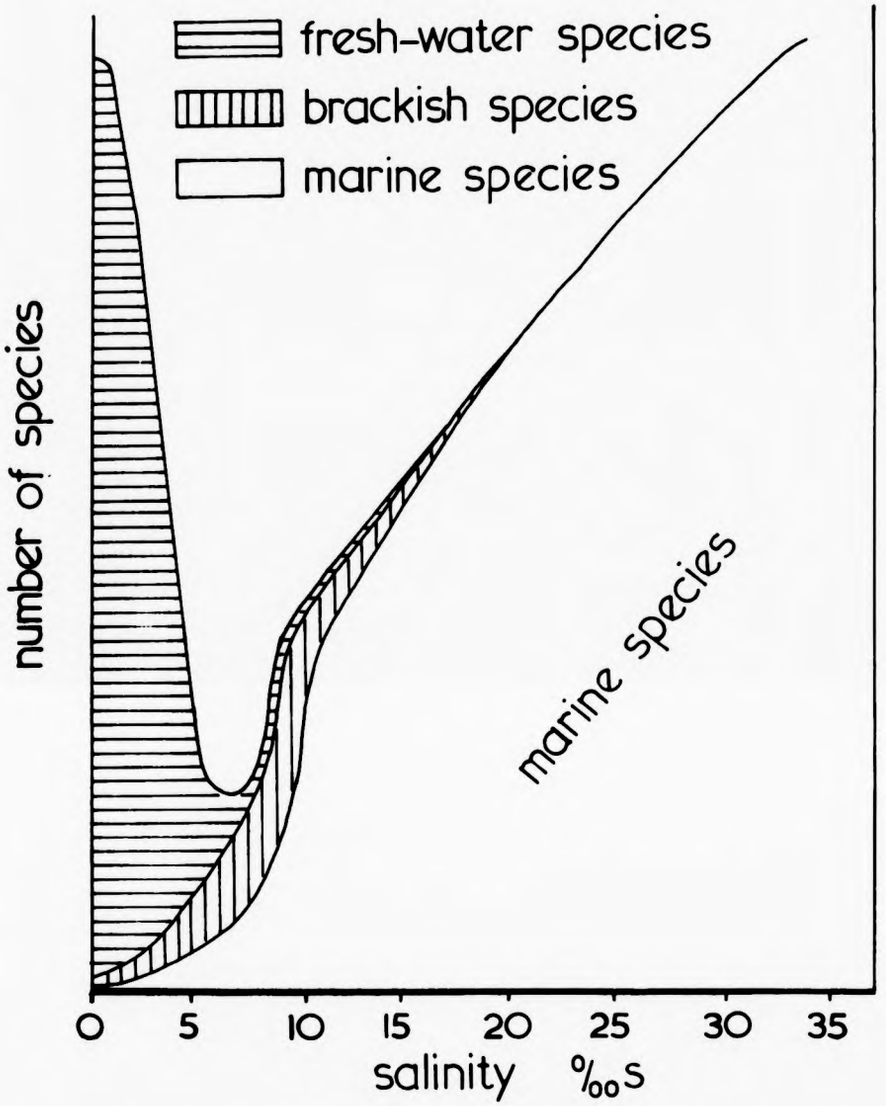

Fig. 32. Number of species in relation to salinity (after Remane, 1934.)

interpreted as indicating that the more permeable insects (particularly some of the chironomids) are unable to cross the 15°/ooS boundary. The data from the Holford brinefield supported this result, and although one Chironomus sp. and Chaoborus sp. were recorded in 14.4°/ooCl (24.2°/ooS) in Pond 8, at higher salinities the fauna became very restricted (tables 26 and 37, pp.139 &176) being characterised by such species as Nemotelus notatus, Aedes detritus, Ephydridae and Ceratopogonidae. These holeuryhaline forms, apart from possessing a mechanism for hypotonic regulation, have achieved almost total independence from the environment by virtue of great reductions in permeability (Beadle 1939, Ramsay 1950, 1951, Nemenz 1960, Sutcliffe 1959, 1960a) and have been recorded from a variety of hypersaline waters from all over the world.

The decline in number of species at salinities of around 15°/oo where halophye 2° species begin to disappear, may therefore signify another natural boundary in chloride-based inland saline waters.

Changes in the total number of individuals.

The decline in total number of species was accompanied by an initial decline in the total number of individuals at both Crow Brook and the Trent and Mersey Canal, though at the higher salinities population density increased and this was presumably due to a response in the halophye 2° component to the elimination of limnobiont competitors and predators. In Crow Brook (figure 9, p46) total number of individuals was lowest at station 8 ,(5.98°/ooS), and this is significant as it suggests that the faunal changes at the upper oligohalobic boundary are not generated by ecological factors but by physiological ones, as numbers of competitors, and probably predators, are also low at this point.

The salinity at which paucity of numbers occurs in the Trent and Mersey Canal is rather lower, but as discussed in Chapter IV this probably results from the low salinity stretch being downstream from the high salinity one.

At salinities greater than 6°/ooS, total numbers of individuals increased again and as these belonged to but a few species, the index of diversity (α) was typically below 4 and frequently below 3 (tables 9 and 24, pp 67 & 125).

Various species apparently proliferated in numbers in different parts of the salinity spectrum. In Crow Brook Gammarus duebeni became abundant between 6°/ooS and at least 7°/ooS. At Aldersey Chironomus riparius and Procladius sagittalis were present in prolific numbers at station 1, but not in the Spore and therefore have a probable salinity optimum around 11°/ooS but not as high as 16°/ooS. Ceratopogonidae were prolific at both sites and their salinity optimum must encompass 11°/ooS and 16°/ooS. Ephydra riparia was numerous at both Aldersey Spore and Lower Wych where it accounted for most of the standing crop, but not at less saline sites. The species must therefore become prolific in salinities in excess of about 15°/ooS. On the Holford brinefield Aedes detritus was present in massive numbers at 40°/ooS.

It seems then, that although the presence of a particular species can be taken as an indication of the salinity, the number of individuals can give a more detailed indication of salinity conditions. A general rule appears to be that where the index of diversity (α) is below 4 in a saline habitat, total salinity probably exceeds 6°/oo. Although additional surveys of inland saline waters are desirable, it appears from the data collated here that where a species is represented in prolific numbers (contributing as much as 95% of individuals to the standing crop) a sequence as given in the following scheme might be expected with progressively higher salinities.

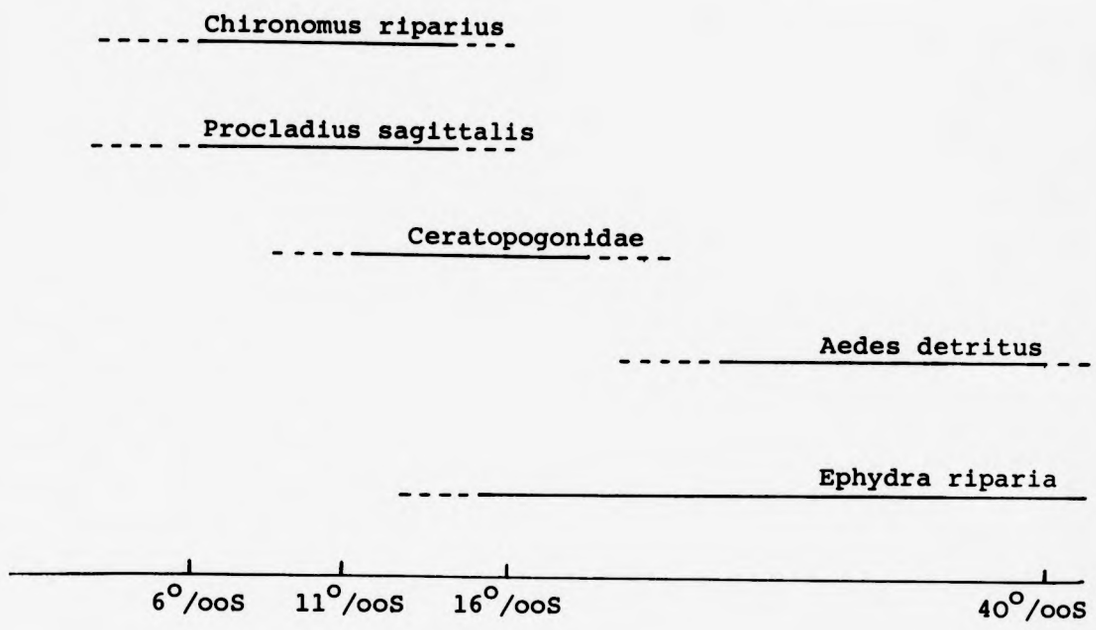

Figure 33. Range of salinities at which species become prolific in numbers.

The biological assay of chloride based inland saline waters.

The problems of assessing and describing the severity of a saline environment have already been mentioned. Whilst mean salinity may give a good indication of the harshness of the salinity regime, the amplitude, frequency and duration of fluctuations in salinity must also contribute to its effect on the fauna. Coefficients of variability which combine either the standard deviation or the range of salinity fluctuations with the mean salinity (den Hartog, 1964) showed no discrimination between stations in Crow Brook, even though faunal changes indicated a considerable increase in the severity of the environment. It therefore became apparent that an assay of the effects of any salinity regime must have a biological basis and the matrix of halobic indices given in table 8, p66 was constructed.

Primarily, a sequence of changes in the major faunal components with increasing salinity was listed along the ordinate of the matrix and the sensitivity of this sequence was improved by specifying relative abundance of each category. The assumption was made that this sequence of changes would remain the same whether the limiting factor was salinity *per se* or poikilohalinity. Thus as a saline environment became more severe, the faunal analysis would be expected to match categories progressively further down this sequence.

A second parameter was introduced along the abcissa of the matrix which is was hoped would discriminate between saline environments with rich faunas and those with impoverished faunas even though their faunas may contain representatives of the major faunal components in identical ratios. Number of species present was used for this parameter and it was assumed that this would decline with harshness of the environment.

An arbitary numerical system could then be inserted into the matrix whereby progressively harsher environments would receive progressively lower halobic indices.

Although the matrix is based on faunal data from Crow Brook and would therefore be expected to rank the stations in order of salinity, it proved valid when applied to the faunas of other sites. Table 43 lists all sites in order of decreasing halobic indices and it becomes apparent that for a given type of habitat such as the brine pits and springs, or the Trent and Mersey Canal, the stations fall into the appropriate salinity sequence (station 12 of the Trent and Mersey Canal being the only exception).

Overall ranking of sites by the halobic index gives a sequence which corresponds well with that of the absolute salinities, and discrepancies only confirm the value of a biological method of assay. Crow Brook generally ranked higher for a given salinity

Table 43. Localities ranked according to their halobic indices (mean salinities compared).

	Halobic index	Mean Salinity (°/ooS)
Crow Brook, station 1.	20	0.11
Crow Brook, station 2.	20	0.20
Trent and Mersey, station 1.	19	0.12
Trent and Mersey, station 2.	19	0.11
Crow Brook, station 3.	19	2. 6
Crow Brook, station 4.	19	2. 7
Trent and Mersey, station 3.	18	0.10
Trent and Mersey, station 4.	18	0.11
Trent and Mersey, station 5.	18	0.12
Pasturefields.	18	1. 4
Crow Brook, station 5.	18	3. 6
Crow Brook, station 6.	18	3. 7
Crow Brook, station 7.	15	5. 3
Trent and Mersey, station 15.	10	3. 4
Trent and Mersey, station 14.	10	4. 4
Aldersey, station 2.	10	5. 2
Crow Brook, station 8.	8	6. 0
Shrewbridge spring.	8	7. 7
Crow Brook, station 10.	7	6. 4
Trent and Mersey, station 13.	6	4. 5
Trent and Mersey, station 11.	6	4. 8
Trent and Mersey, station 10.	6	5. 7
Trent and Mersey, station 9.	6	6. 0
Trent and Mersey, station 8.	6	6. 9
Crow Brook, station 9.	6	5. 6
Crow Brook, station 11.	6	7. 0
Aldersey, station 1.	6	11. 2
Trent and Mersey, station 12.	5	4. 4
Trent and Mersey, station 7.	5	8. 4
Aldersey Spore.	5	16. 1
Trent and Mersey,station 6.	3	7. 8
Lower Wych Brine Pit.	3	96. 4

than other sites and this indicates that fluctuations in salinity were probably not a critical factor. The richness of the fauna was also probably maintained by invertebrate drift which would replenish invertebrate stocks. The Trent and Mersey Canal generally rated lower than the other sites for a given salinity and it has already been mentioned that the poverty of the fauna is probably attributable to the downstream position of the low salinity stretch and the presence of other pollutants.

This matrix of halobic indices, with further refinement, could prove to be a valuable tool for biological monitoring. Not only does the index provide a method by which comparison can be made between poikilohaline environments of various types and stable environments, it also enables direct assays to be made. The biological consequences of a brine pollution could, for example, be interpreted to the layman by use of the halobic index, rather than by detailed verbal descriptions of the faunal changes and the interpretation put upon them.

The classification of inland saline waters.

Hedgpeth (1959) considered that the quantitative ecology of inland mineral waters had been greatly neglected and he pointed out the eventual need for a biologically based system of classification. The series of investigations reported here are a preliminary step toward achieving this goal and the validity of the halobic index indicates that the various ecological changes discussed earlier in this chapter are quantifiable trends and have some value for the classification of inland saline waters. At the Symposium on the Classification of Brackish waters (Anon, 1958) several contributors strongly urged that inland saline waters should not be included in a marine-brackish system of classification on the grounds of differences in ionic composition and also of faunal origin and affinity. While it is certainly true that at the higher salinities the faunas of marine-brackish and inland saline waters are very different, the investigations reported here give no indication that

a common system of classification cannot be used for the lower salinities. It is therefore suggested that the limnetic zone ($< 0.5^{o}/oo$S) and the oligohaline zone ($\pm$ 0.5 to $\pm$ $5^{o}/oo$S) as defined by the Symposium be retained in a classification of chloride based inland saline waters. In both types of habitat the limnetic zone is characterised by a rich freshwater fauna which includes stenohaline species and within the zone there is no decline in number of species. In the oligohaline zone however, the number of limnobiont species declines with increasing salinity as the less euryhaline forms are eliminated, and reaches a minimum at the upper boundary of the zone. The total number of individuals also declines progressively through the oligohaline zone and the index of diversity (α) falls from around 7 or 8 to about 4. Species typical of this zone are the euryhaline species of the first, second and third degree and the halophye species of the first degree that are mentioned earlier in this chapter.

As in marine brackish waters, the reduction in number of limnobiont species is apparently not attributable to ecological factors but to physiological ones and this also justifies the use of the upper oligohalobic boundary in classifying both types of saline water. Beadle (1959) confirmed that this is probably an important physiological boundary. He pointed out that 4-$6^{o}/oo$S is the level of the most dilute body fluids found in freshwater animals, and that the mode for blood osmotic pressure is about $5.15^{o}/oo$S. In the present series of investigations the median lethal doses of sodium chloride as established for three stream invertebrates confirm that the tolerance of these species falls within the oligohalobic zone.

At salinities greater than about $6^{o}/oo$, the composition of the faunas of inland mixohaline waters and those of true brackish waters diverge. Whilst marine and brackish water species become increasingly important in true brackish waters (Remane, 1934) it is apparent from the current study and previous ones,that it is the Diptera which are the predominant component of the fauna in inland

saline waters (Baas-Becking, 1928; Hesse, Allee and Schmidt, 1937; Thienemann, 1954; Hedgpeth, 1959, Beadle 1959, 1969).

The distinction between the faunas of the two types of saline water at high salinities has also been made by earlier workers. Hedgpeth (1951) noticed that whilst marine hypersaline lagoons had an essentially marine fauna, inland waters of the same salinity and composition were characterised by insect larvae (eg. Ephydra spp) and also phyllopod and copepod Crustacea. Beadle (1959) attributed the difference to absence of large predators and to salinity fluctuations caused by precipitation and evaporation. Hedgpeth (1956) thought that predation by fish was important in eliminating the characteristic inland saline fauna from marine brackish habitats and Segerstråle (1959) agreed that it was ecological factors which prevented Ephydra sp. from occupying true brackish waters.

On the grounds of this dimorphism of the fauna at the higher salinities in the two types of waters, inland saline waters were excluded from the brackish water system developed by the Venice Symposium (Anon, 1958) and the classification of inland saline waters was consequently neglected. As there have been very few quantitative studies of inland saline waters, little attempt has been made to develop a system for their classification, even in recent years. However, the data collated in this report now make it possible to construct a preliminary classification which it is hoped will prove appropriate for all chloride-based inland saline waters.

The applicability of the limnetic and oligohaline zones to inland saline waters and the validity of the 5-6^{o}/ooS boundary have already been mentioned. The Diptera became an important component at all sites where salinity was greater than 6^{o}/oo (table 31,p149) but as mentioned earlier in this chapter and in chapter V, there appeared to be another natural salinity boundary at around 15-16^{o}/ooS. While limnobiont chironomids made a significant contribution to the

faunas at Shrewbridge (7.7°/ooS), and Aldersey station 1 (11.2°/ooS), their numbers were greatly reduced at Aldersey Spore (16.1°/ooS). The mixohaline species which have freshwater origins also appear to occupy the same range of salinities in inland waters. Limnephilus affinis was found in salinities up to 14.4°/ooS and breeding populations of Sigara stagnalis and S.selecta were found up to 14°/ooS.

There therefore seem to be good biological grounds on which a zone of the approximate range 5-6°/ooS - 16°/ooS could be utilised in a classification system. Although the zone corresponds approximately with the mesohaline zone (± 5°/ooS - ± 18°/ooS) in the "Venice System" (Anon 1958) the term will be avoided to prevent misunderstanding. Furthermore, as inland saline waters may vary in their severity, not only as a result of salinity per se, but also as a result of poikilohalinity, precise salinity limits and strict terminology should be avoided.

The problem can be overcome by describing the limits of the zones in terms of the halobic index which makes allowance for the effects of variability of salinity. The various zones of the classification have been described in terms of their faunal components rather than by terminology based on chemical parameters, and the zone of the approximate range ± 5-6°/ooS - 16°/ooS which includes all sites with halobic indices between 8 and 5-6 has been named the 'zone of limnobiont chironomids'. The reservation must be made that those genuine mixohaline chironomids which have developed special abilities for life in saline water (Chironomus aprilinus, C.salinarius and Halocladius varians) must be excluded.

For habitats with halobic indices less than 5 (salinity greater than 15-16°/ooS), the system became more hypothetical as Aldersey Spore and Lower Wych pit were the only sites at which more extreme conditions were recorded. One feature which had to be considered was that there are a number of genuine brackish water species which occur in inland saline waters where other factors are not limiting, and it seems probable that species like Gammarus zaddachi,

Table 44. System for the classification of chloride-based inland saline waters.

zone			halobic index.	approximate salinity limits (‰S).	indicator groups and species.
Zone of stenohaline limnobionts (Limnetic zone)			20	0 - 0.5	Hydropsyche pellucidula (in running waters only)
Zone of euryhaline limnobionts (Oligohalobic zone)			19 -8	0.5 - 5.6	All limnobiont species excluding Diptera mentioned below and Nais elinguis and Lumbricillus reynoldsdsoni
Zone of limnobiont chironomids			8 - 6/5	5-6 - 16	Chironominae excluding genuine brackish water species. Limnephilus affinis, nymphs of Sigara stagnalis and S.selecta. Procladius sagittalis.
Ephydrid zone	Zone of tolerant and halobiont Diptera.	Zone of genuine brackish water species.	6/5 -?4	16 - ?35-40	Gammarus zaddachi G.duebeni (where other factors permit). Chironomus aprilinus C.salinarius Halocladius varians
			6/5 - ?3	16 - ?100	Dolichopodidae Psychodidae Scatophagidae Sepsidae Ceratopogonidae Tabanidae Syrphidae
			3	100	Ephydra riparia Nemotelus notatus Atissa pygmaea

G.duebeni and the genuine brackish water chironomids would occupy a zone with an upper limit where salinities just exceeded those of seawater. This zone is also occupied by the tolerant and halobiont Diptera in static waters (eg. Diptera associated with dung), but they probably accept higher salinities than the genuine brackish water species and may be found in salinities up to 100°/oo (eg. Aedes detritus; Beadle,1939). At greater salinities only the holeuryhaline Diptera which have powerful osmoregulatory abilities and a high impermeability, would remain (eg. Ephydra riparia).

The suggested system of classification for chloride-based inland saline waters as derived here, and which incorporates the halobic indices from table 43,(p220),is presented in table 44.

Although the classification is, on the whole, empirically based, the boundaries of the higher salinity zones have been treated somewhat theoretically, and it is hoped that as more inland saline waters are studied quantitatively and in detail, the classification will be consolidated.

SUMMARY

1. The ecological changes accompanying increases in salinity in inland waters are discussed.

2. Changes in the composition of the fauna include the progressive elimination of euryhaline species of the first, second and third degree, and a rise and fall in the abundance of halophye and brackish water species. At extreme salinities only holeuryhaline species remain. Groups and species which appeared to be good indicators of salinity were identified and the Corixidae, in particular, were identified as a sensitive group.

3. The total number of species generally declined with increasing salinity, though the halophye reaction of many Diptera accounted for a good diversity of species being maintained at salinities up to 16°/ooS. The majority of euryhaline limnobionts were

eliminated by 6°/ooS, a trend which is paralleled in marine-brackish waters.

4. Total numbers of individuals decreased as salinities reached 6°/ooS, but recovered at greater salinities as members of tolerant species proliferated in the absence of competitors. The index of diversity therefore generally fell with increasing salinity and values below 4 indicated salinities greater than 6°/ooS.

5. The advantages of a biological method of assay of salinity in chloride-based inland waters were discussed. The matrix of halobic indices developed in the present study gave a consistent evaluation of all the sites investigated and its value as a biological tool was confirmed.

6. An empirically based system of classification applicable to inland saline waters was postulated. Whilst zones equivalent to the limnetic and oligohaline zones of the "Venice System" for marine-brackish waters (Anon 1958) were retained for inland waters, it was necessary to develop a separate system for salinities greater than c.6°/ooS as the fauna was distinctive and bore little resemblence to that of brackish waters.

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XI APPENDICES

APPENDIX I

Comparison of hydrogen ion concentrations in freshwater and saline reaches of Crow Brook.

Null hypothesis: The pH at station 1 of Crow Brook does not differ significantly from the pH at station 11.

$$t = \frac{\bar{x}_{11} - \bar{x}_1}{\sqrt{\left(\frac{\sigma_1^2}{n_1} + \frac{\sigma_{11}^2}{n_{11}}\right)}}$$

where $\bar{x}$ is mean pH at the given station.

σ is standard deviation of pH at the given station.

$t = 0.7112$

With df = 20, this value is not significant at the 5% level and the null hypothesis must be accepted.

APPENDIX 2.

Comparison of salinities at station 2 and station 5 of the Trent and Mersey Canal.

Null hypothesis: Salinity conditions at station 2 of the Trent and Mersey Canal were the same as those at station 5.

x_2	x_5
44	77
64	113
72	78
83	109
65	87
57	105
44	48
65	54
44	32
81	54
61	85
94	48
58	65

$\bar{x}_2 = 63.9$ $\qquad$ $\bar{x}_5 = 73.5$

$\sigma_{x_2} = 15.5$ $\qquad$ $\sigma_{x_5} = 25.8$

$\frac{\sigma^2_{x_2}}{N} = 51.2$ $\qquad$ $\frac{\sigma^2_{x_5}}{N} = 18.48$

$\sigma_d^2 = 69.7$

$\sigma_d = 8.34$

$t = \frac{\bar{x}_2 - \bar{x}_5}{\sigma_d} = 1.15$

Considering df = 24, then t = 1.15 is not significant at the 5% level and the null hypothesis is therefore accepted.

APPENDIX 3

Computation of the degree of association between three species of Gammarus using Kendall's tau (Kendall 1962).

Null hypothesis 1: There is no correlation between the distribution of Gammarus duebeni and Gammarus tigrinus.

Station number	x_d	R_d	x_t	R_t
9	9	3.5	19	4
10	9	3.5	1	2
11	25	5	1	2
12	6	2	1	2
13	32	6	29	5
14	137	7	889	7
15	3	1	44	6

Comparing ranks of abundance for the two species:-

S = (1-5) + (3-0) +(2-0) + (2-0) + (1-0).

= -4+3+2+2+1

= 4

$$T = \frac{2S}{N(N-1)}$$

$$= \frac{8}{42}$$

= 0.1905 p = 0.335

The null hypothesis is accepted.

Null hypothesis 2: There is no correlation between the distribution of Gammarus duebeni and Gammarus zaddachi.

Station number	x_d	R_d	x_z	R_z
9	9	3.5	9	3
10	9	3.5	222	7
11	25	5	172	6
12	6	2	62	5
13	32	6	32	4
14	137	7	8	2
15	3	1	3	1

Comparing ranks of abundance for the two species:-

S = (6-0) + (2-3) + (2-1) + (0-3) +(0-2) +(0-1)

= 6-1+1-3-2-1

= 0 p = 0.5

The null hypothesis is accepted.

Null hypothesis 3: There is no correlation between the distribution of Gammarus tigrinus and Gammarus zaddachi.

Station number	t	R_t	z	R_z
9	19	4	9	3
10	1	2	222	7
11	1	2	172	6
12	1	2	62	5
13	29	5	32	4
14	889	7	8	2
15	44	6	3	1

Comparing the ranks of abundance for the two species:-

S = (0-4) + (0-4) + (1-2) + (0-2) +(1-0) + (0-4)

= -4-4-4-1-2+1

= -14

Correcting for ties (Table Q, Siegel 1962*).

S = -15.12

T = -0.6668 p= 0.015

The null hypothesis is rejected at the 2% level of significance.

*Siegel,S. (1956). Nonparametric statistics for the Behavioural Sciences. New York - McGraw-Hill.

APPENDIX 4

Preparation of artifically hardened water.

The artifically hardened water used in toxicity tests was prepared using three stock solutions. Their compositions were as follows:-

Solution A

530g	anhydrous calcium chloride	in 2 litres deionised water.
47g	sodium chloride	
12.5g	sodium nitrate	
66.4g	potassium nitrate	

Solution B

234g	hydrated magnesium sulphate	in 2 litres deionised water.
125g	anhydrous Na_2SO_4	

Solution C

105g	sodium carbonate	in 2 litres deionised water.

To harden deionised water to $250mgl^{-1}$ $CaCO_3$, 1ml of solution A, 1ml of solution B and 5ml of solution C were added to each litre of water.

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